Bioactive Constituents from *Dracocephalum subcapitatum* (O. Kuntze) Lipsky

Soodabeh Saeidnia[a,*], Ahmad Reza Gohari[a], Michiho Ito[b], Fumiyuki Kiuchi[c], and Gisho Honda[b]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Mazandaran University of Medical Sciences, PO Box 48175-861, Sari, Iran. Fax: +98-151-3254060. E-mail: soodabehsaeidnia@hotmail.com
[b] Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan
[c] Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, Ministry of Health, Labour and Welfare, Tsukuba, Ibaraki, 305-0843, Japan

* Author for correspondence and reprint requests

Z. Naturforsch. **60 c,** 22–24 (2005); received August 4/September 14, 2004

From an EtOAc extract of *Dracocephalum subcapitatum*, five flavonoids, calycopterin, xanthomicrol, isokaempferide, luteolin and apigenin, together with five terpenoids, oleanolic acid, ursolic acid, geranial, neral and limonene-10-al, were isolated. Among them, citral and limonene-10-al were the most effective components against epimastigotes of *Trypanosoma cruzi*, the parasitic agent of Chagas disease.

Key words: Dracocephalum subcapitatum, Labiatae, Limonene-10-al

Introduction

Dracocephalum is a genus belonging to the Labiatae family and is found abundantly in Central Asia, Iran, Turkey and Europe (Rechinger, 1986; Mozaffarian, 1996). These herbaceous plants have been used in traditional medicine for stomach and liver disorders, headache and congestion (Mirheydar, 1995). Recently, some trypanocidal diterpenoids were isolated from *D. komarovi* (Uchiyama *et al.,* 2003; 2004). We have also reported the isolation of trypanocidal flavonoids and terpenoids from *D. kotschyi* (Gohari *et al*., 2003; Saeidnia *et al*., 2004b) and their activity test against epimastigotes of *Trypanosoma cruzi*, the parasitic agent of Chagas disease (Nogueda-Torres *et al*., 2001). Among eight Persian species of this genus *D. subcapitatum* (O. Kuntze) Lipsky is found at limited area in the north-east of Iran (Rechinger, 1986). Literature reviews show that nothing has been reported on phytochemical analysis of this plant. We present here the isolation and identification of the constituents from *D. subcapitatum* and their trypanocidal activity.

Material and Methods

General

Melting points were determined on a Yanagimoto micro melting point apparatus. ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM-LA500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given as δ values.

Plant material

Aerial parts of *Dracocephalum subcapitatum* (O. Kuntze) Lipsky were collected from the northern part of Khorasan prefecture, Iran in May 2002. Mr. I. Mehregan (Shaheed Beheshti University of Medical Sciences, Tehran, Iran) identified the plant species. A voucher specimen was deposited at the Experimental Station of Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

Extraction and isolation

Dried aerial parts of *D. subcapitatum* (350 g) were cut into small pieces and successively extracted with EtOAc and MeOH at room temperature overnight to obtain EtOAc (15 g) and MeOH (10 g) extracts. These extracts were tested against epimastigotes of *T. cruzi*. The active EtOAc extract (4 g) was submitted to silica gel column chromatography (CC) with hexane/EtOAc (19:1, 0:1 v/v) and MeOH as eluents to give five fractions (A–E). Among them, fractions B (10 mg), C (400 mg) and D (2.0 g) were active against *T. cruzi*. Fraction B was compound **1**. Fraction C was fractionated with hexane/$CHCl_3$ (1:1, 2:3 v/v) to afford three parts (C_1–C_3). Fraction C_1

0939–5075/2005/0100–0022 $ 06.00

was compound **1** (20 mg). Fraction C_3 was chromatographed twice with hexane/EtOAc (19:1, 9:1 v/v) on silica gel to give compounds **2** (20 mg) and **3** (10 mg). From fraction D, compound **4** (20 mg) and compound **5** (30 mg) were obtained by silica gel CC with hexane/EtOAc (19:1), hexane/acetone (8:2) and then $CHCl_3$/EtOAc (9:1 v/v). Further purification of compound **5** was performed with $CHCl_3$/EtOAc (19:1). The more polar part of fraction D (1.2 g), remaining after the separation of compounds **4** and **5**, was chromatographed on silica gel with hexane/EtOAc (6:4) to obtain three parts (D_1–D_3). From D_2 (800 mg), compounds **6** (10 mg), **7** (15 mg) and **8** (30 mg) were isolated on Sephadex LH-20 with $CHCl_3$/MeOH (7:3) as a solvent system. Fractionation of D_3 (400 mg) on silica gel (benzene/EtOAc, 3:1) and purification of the separated fractions with Sephadex LH-20 (MeOH) resulted in compounds **9** (35 mg) and **10** (18 mg).

Trypanocidal assay

Epimastigotes (prepared from Juntendo University, Japan) of *T. cruzi* (Tulahuen strain) were kept in GIT medium (Wako, Tokyo) supplemented with hemin (12.4 μM; Wako). The epimastigotes in GIT medium (10 μl) were incubated with a test sample dissolved in EtOH (5 μl) and autoclaved salin (185 μl). After 24 h of incubation, the movement of epimastigotes was observed under a microscope. We assumed that the immobilized and ball-shaped organisms were dead. Five determinations for minimum lethal concentration (MLC, concentration on which, all epimastigotes were dead) of each compound were performed. The negative control used, contained ethanol in the same proportion utilized to dissolve the compounds. Also, gentian violet was used as a positive control (MLC = 6.3 μM) (Kiuchi *et al.,* 2002).

Results and Discussion

Dried aerial parts (leaves, flowers and stems) of *D. subcapitatum* were successively extracted with EtOAc and MeOH. Only the EtOAc extract showed *in vitro* trypanocidal activity (MLC = 50 μM). Therefore, we fractionated this extract and obtained 10 pure compounds (**1**–**10**). The identification of these compounds was carried out by comparison of their spectral data (^{1}H and ^{13}C NMR spectra) with previous reports (Table I).

Table I. *In vitro* activity of the isolated constituents from *Dracocephalum subcapitatum* against the epimastigotes of *T. cruzi.*

Compound	MLC* [μM] Mean ± SD	References
Limonene-10-al (**1**)	3.1 ± 0.3	Saeidnia *et al*., 2004b
Geranial (**2**)	3.1 ± 0.3	Bohlmann *et al*., 1975
Neral (**3**)	3.1 ± 0.0	Bohlmann *et al*., 1975
Oleanolic acid (**4**)	6.2 ± 0.8	Srivastava and Jain, 1989
Ursolic acid (**5**)	6.2 ± 0.3	Alves *et al*., 2000
Calycopterin (**6**)	>400	El-Ansari *et al*., 1991
Xanthomicrol (**7**)	>400	El-Ansari *et al*., 1991
Isokaempferide (**8**)	70.0 ± 17.5	Gohari *et al*., 2003
Apigenin (**9**)	30.0 ± 15.0	Wawer and Zielinska, 2001
Luteolin (**10**)	>400	Flamini *et al*., 2001

* Minimum lethal concentrations of the separated compounds; five determinations for each concentration were tested and immobilized organisms were assumed to be dead.

These compounds were limonene-10-al (**1**), geranial (**2**), neral (**3**), oleanolic acid (**4**), ursolic acid (**5**), calycopterin (**6**), xanthomicrol (**7**), isokaempferide (**8**), apigenin (**9**) and luteolin (**10**).

All the pure compounds were tested against epimastigotes of *T. cruzi*. The average of five MLC determinations for the active constituents are reported in Table I. Highly methoxylated flavonoids, calycopterin and xanthomicrol, showed no activity even at 400 μM. Among these known compounds, citral (geranial and neral) and limonene-10-al were the most effective components against epimastigotes of *Trypanosoma cruzi*. In addition *D. subcapitatum, D. feotidum* and *D. kotschyi* contain a C10 aldehyde derivate of limonene (Duetze *et al.,* 2003; Saeidnia *et al.*, 2004b). Phylogenetic analysis of *Dracocephalum* species showed that *D. subcapitatum* has very close relationship to *D. kotschyi* compared to other species (Saeidnia *et al.,* 2004a). The results obtained from this study, together with our previous research on chemical constituents of *D. kotschyi* (Gohari *et al.,* 2003; Saeidnia *et al*., 2004b), showed chemotaxonomical similarity of these two species (*D. kotschyi* and *D. subcapitatum*), to support their close phylogenetic relationship.

Acknowledgements

The authors wish to thank Mr. Iraj Mehregan for collection and identification of plant material.

Agrawal P. K. (1989), Carbon-13 NMR of Flavonoids. Elsevier Science, Amsterdam.

Alves J. S., de Castro J. C. M., Freire M. O., da-Cunha E. V. L., Barbosa-Filho J. M., and de Silva M. S. (2000), Complete assignment of the ^{1}H and ^{13}C NMR spectra of four triterpenes of the ursane, artane, lupane and friedelane groups. Mag. Res. Chem. **38**, 201–206.

Bohlmann F., Zeisberg R., and Klein E. (1975), ^{13}C-NMR Spektren von Monoterpenen. Org. Mag. Res. **7**, 426–432.

Duetze W. A., Bouwmeester H., and Van Beilen J. B. (2003), Biotransformation of limonene by bacteria, fungi, yeasts and plants. Appl. Microbiol. Biotechnol. **61**, 269–277.

El-Ansari M. A., Barron D., Abdalla M. F., Saleh N. A. M., and Le Quere J. L. (1991), Flavonoid constituents of *Stachys aegyptica*. Phytochemistry **30**, 1169–1173.

Flamini G., Antognoli E., and Morelli I. (2001), Two flavonoids and other compounds from aerial parts of *Centaurea bracteata* from Italy. Phytochemistry **57**, 559–564.

Gohari A. R., Saeidnia S., Matsuo K., Uchiyama N., Yagura T., Ito M., Kiuchi F., and Honda G. (2003), Flavonoid constituents of *Dracocephalum kotschyi* growing in Iran and their trypanocidal activity. Nat. Med. **57**, 250–252.

Harborne J. B. (1993), The Flavonoids: Advances in Research since 1986. Chapman and Hall, London, pp. 450–451.

Kiuchi F., Itano Y., Uchiyama N., Honda G., Tsubouchi A., Nakajima-Shimada J., and Aoki T. (2002), Monoterpene hydroperoxides with trypanocidal activity from *Chenopodium ambrosoides*. J. Nat. Prod. **65**, 509–512.

Mirheydar H. (1995), Maaref Giahi, vol. 2. Daftare Nashre Farhange Eslami (Publisher), Tehran, pp. 170–176.

Mozaffarian V. (1996), A Dictionary of Iranian Plant Names. Farhange Moaser (Publisher), Tehran, pp. 192–193.

Nogueda-Torres B., Rodriguez-Paez L., Ramirez I. B., and Ramirez C. W. (2001), Trypanocidal activity of isopropyl salicylaldehyde and 4-isopropyl salicylic acid on *Trypanosoma cruzi*. Rev. Lat. Microbiol. **43**, 1–6.

Rechinger H. (1986), Flora Iranica, Labiatae, vol. 150. Akademische Druck- und Verlagsanstalt, Graz, Austria, pp. 218–230.

Saeidnia S., Gohari A. R., Ito M., and Honda G. (2004a), Comparison of some *Dracocephalum* species by phylogenetic and chemotaxonomic analysis. Int. J. Biol. Biotech. **1**, 267–270.

Saeidnia S., Gohari A. R., Uchiyama N., Ito M., Honda G., and Kiuchi F. (2004b), Two new monoterpene glycosides and other trypanocidal terpenoids from *Dracocephalum kotschyi*. Chem. Pharm. Bull. **52**, 1249–1250.

Srivastava S. K. and Jain D. C. (1989), Triterpenoid saponins from plants of Araliaceae. Phytochemistry **28**, 644–647.

Uchiyama N., Kiuchi F., Ito M., Honda G., Takeda Y., Khodzhimatov O. K., and Ashurmetov O. A. (2003), New icetexane and 20 norabietane diterpenes with trypanocidal activity from *Dracocephalum komarovi*. J. Nat. Prod. **66**, 128–131.

Uchiyama N., Ito M., Kiuchi F., Honda G., Takeda Y., Khodzhimatov O. K. and Ashurmetov O. A. (2004), A trypanocidal diterpene with novel skeleton from *Dracocephalum komarovi*. Tetrahedron Lett. **45**, 531–533.

Wawer I. and Zielinska A. (2001), ^{13}C CP MAS NMR of flavonoids. Magn. Reson. Chem. **39**, 374–380.