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Trypanocidal constituents of *Dracocephalum komarovi*

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Abstract—Trypanocidal constituents of *Dracocephalum komarovi* were investigated. Under guidance of the in vitro trypanocidal activity against epimastigotes of *Trypanosoma cruzi*, the causative agent of Chagas' disease, two new diterpenes, dracocequinones A (**1**) and B (**2**), and two known triterpene acids, ursonic acid and ursolic acid, were isolated as trypanocidal constituents, in addition to previously reported diterpenes, cyclocoulterone (**4**), komaroviquinone (**5**), dracocephalone A (**6**) and komarovispirone (**7**). Furthermore a new diterpene, komarovinone A (**3**), was isolated, together with four known terpenes. Among these compounds, komaroviquinone (**5**) showed the most potent activity with minimum lethal concentration of 0.4 μM. Structure elucidation of the new diterpenes **1–3** was described.

1. Introduction

Chagas' disease is a major public health problem endemic in Central and South American countries, with 18–20 million infected people, 25% of the human population at risk of infection, ca. 200,000 new cases, and 21,000 deaths per year.[1] Its causative agent is *Trypanosoma cruzi*, a parasitic protozoan transmitted to mammalian host by blood-sucking triatomine bugs. *T. cruzi* undergoes three main developmental stages during its life cycle, that is, the replicative epimastigote form in insect vectors and the trypomastigote and amastigote forms in mammalian hosts. Non-dividing and infective trypomastigotes circulate in the blood with their free flagellum before invading host cells, preferably muscle cells, where they lose their flagellum to differentiate into replicative amastigotes.[2] Infections by *T. cruzi* result in a life-threatening, acute and/or chronic disease with severe cardiac complications. This situation is worsened by the lack of effective vaccines, undesirable side effects of anti-chagasic drugs in use such as nifurtimox and benznidazole, and the emergence of parasite resistance to these drugs. Therefore, development of new chemotherapeutic agents is urgently needed.

The genus *Dracocephalum* is an annual or perennial herb of the Labiatae family, occurring widely in Southern Europe and temperate Asia. Some of its species are used as an astringent and a carminative,[3] and are reported to show antihyperlipidemic effect,[4] immunomodulatory effect[5] and antinociceptive effect.[6] *Dracocephalum komarovi* Lipsky is a perennial semishrub that grows at around 2300–3600 m above sea level in the West Tien Shan mountain system.[7] It is called 'buzbosh' in Uzbekistan and the local people use the aerial parts in a tea to cure various disorders such as inflammatory diseases and hypertony. During our screening of medicinal plants of Uzbekistan for trypanocidal activity, this plant showed trypanocidal activity, and we previously reported the isolation of four new diterpenes, cyclocoulterone (**4**), komaroviquinone (**5**), dracocephalone A (**6**)[8] and komarovispirone (**7**)[9] from the hexane and EtOAc extracts. In this paper, we report a full account of the elucidation of trypanocidal constituents of *D. komarovi*, including the isolation and structure elucidation of three new diterpenes.

2. Results and discussion

Dried whole plants of *D. komarovi* were extracted as described previously[8] (Scheme 1). The hexane and EtOAc

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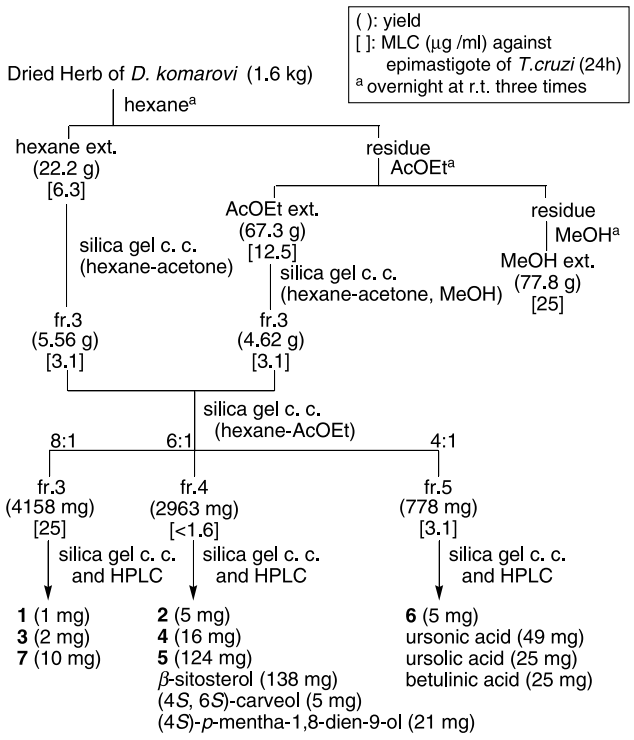


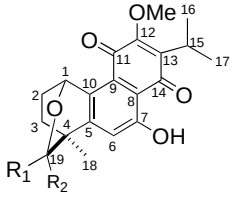
Scheme 1.

extracts were fractionated by silica gel column chromatography using hexane–acetone and MeOH as eluents. The fractions that were eluted with hexane–acetone (6/1) from the hexane extract, and hexane–acetone (8/1) from the EtOAc extract, showed strong in vitro trypanocidal activity against epimastigotes of *T. cruzi*. These fractions were further separated by silica gel column chromatography and HPLC to give seven new compounds **1** (1 mg), **2** (5 mg), **3** (2 mg), **4** (16 mg), **5** (124 mg), **6** (5 mg) and **7** (10 mg), together with ursonic acid,[10] ursolic acid,[11] betulinic acid,[12] β-sitosterol,[13] (4*S*,6*S*)-carverol[14] and (4*S*)-*p*-mentha-1,8-diene-9-ol.[15] The structures of compounds **4** (cyclocoulterone), **5** (komaroviquinone), **6** (dracocephalone A), **7** (komarovispirone) were reported previously.[8,9] The known compounds were identified by comparisons of the physical and spectroscopic data with those reported.

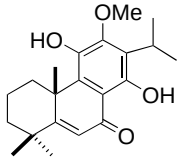
Compound **1** was obtained as an orange oil. The molecular formula $C_{20}H_{22}O_5$ was revealed by high-resolution electron-impact mass spectrum (HREIMS). The presence of a tetra-substituted *p*-benzoquinone moiety (δ_C 112.4, 124.5, 183.4, 159.2, 138.5, 191.3) with methoxy (δ_C 60.9) and isopropyl (δ_C 20.2, 20.4, 24.3) groups, which is similar to that found in komaroviquinone (**5**),[8] was concluded from its ^{13}C NMR and HMBC spectra (Table 1, Fig. 1). However, the chemical shifts of the ring juncture carbons (C-8, δ_C 112.4; C-9, δ_C 124.5) suggested the presence of further conjugation. In fact, HMBC correlations from the chelated hydroxy (δ_H 12.97) and olefin (δ_H 7.12) protons connected this enol system to the *p*-benzoquinone part to form a hydroxy naphthoquinone moiety (Fig. 1). Homo-gated decoupling (HOM) experiments revealed an ^1H–^1H coupling network between an oxymethine proton (δ_H 6.09; δ_C 64.7) and protons of two methylenes (δ_H 1.36, 1.64, 1.80, 2.41). This part structure was connected to the hydroxy naphthoquinone moiety through a quarterly carbon (δ_C 35.4), which also had a methyl (δ_C 18.6) and an oxymethylene (δ_C 71.6) groups. Finally, the two oxygen-bearing carbons (δ_C 64.7 and 71.6) were connected through an ether linkage, because there was only one oxygen atom left in the molecule. Irradiation of the H-6 proton (δ_H 7.12) resulted in a nuclear Overhauser effect (NOE) on H-18 (δ_H 1.34, α-methyl) (Figure 2). Thus, compound **1** was concluded to have the structure indicated, and was named dracocequinone A.

Compound **2** was obtained as an orange oil. This compound showed very similar NMR spectra to those of **1**. However, compound **2** showed no oxymethylene protons corresponding to H-19 in **1**, and instead of the oxygen-bearing carbon at δ_C 71.6 in **1**, compound **2** had an ester carbonyl at δ_C 174.2. This was compatible with its molecular formula $C_{20}H_{20}O_6$ revealed by HRMS. Thus, compound **2** was concluded to be a 19-keto derivative of **1**, and was named dracocequinone B.

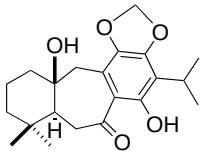
Compound **3** was obtained as a yellow amorphous solid. The ^{13}C NMR spectrum showed very similar chemical shifts for C-1 to C-7 carbons to those of salvinolone (**8**)[16] (Table 1). However, the molecular formula $C_{21}H_{28}O_4$ (HREIMS) together with the ^1H and ^{13}C NMR spectra indicated the presence of an additional methoxy group



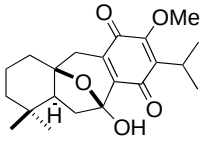
dracocequinone A (**1**): R_1=R_2=H
dracocequinone B (**2**): R_1,R_2= =O



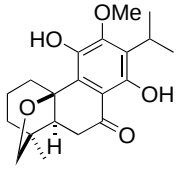
komarovinone A (**3**)



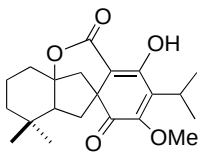
cyclocoulterone (**4**)



komaroviquinone (**5**)



dracocephalone A (**6**)



komarovispirone (**7**)