New Icetexane and 20-Norabietane Diterpenes with Trypanocidal Activity from *Dracocephalum komarovi*

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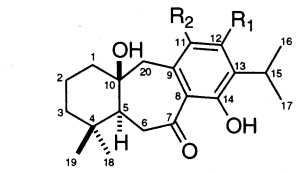
Two new icetexane diterpenes, cyclocoulterone (**1**) and komaroviquinone (**2**), and a novel 20-norabietane diterpene, dracocephalone A (**3**), were isolated from *Dracocephalum komarovi*. Their structures were elucidated by extensive analyses of spectral data. Komaroviquinone (**2**) showed strong trypanocidal activity against epmastigotes of *Trypanosoma cruzi*, the causative agent of American trypanosomiasis, with a minimum lethal concentration of 0.4 μM.

Dracocephalum komarovi Lipsky (Labiatae) is a perennial semishrub that grows at around 2300−3600 m above sea level in the West Tien Shan mountain system.[1] 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. Surprisingly, there have been no previous reports on the constituents of this plant. In our search for trypanocidal compounds in medicinal plants used in Uzbekistan, we isolated new icetexane diterpenes, cyclocoulterone (**1**) and komaroviquinone (**2**), and a 20-norabietane diterpene, dracocephalone A (**3**), from hexane and EtOAc extracts of *D. komarovi*. In this paper, we report the isolation and structure determination of these compounds.

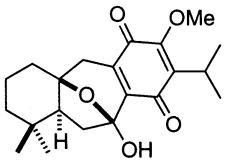
Dried whole plants of *D. komarovi* were successively extracted with hexane and EtOAc at room temperature, and each extract was fractionated by silica gel column chromatography using hexane−EtOAc and MeOH as eluents. The fractions that eluted with hexane−EtOAc (8:1 and 6:1) from the hexane extract, and hexane−EtOAc (6:1) from the EtOAc extract showed strong in vitro trypanocidal activity against epimastigotes of *Trypanosoma cruzi*, the causative agent of Chagas' disease in Central and South America.[2] These fractions were further separated by silica gel column chromatography and HPLC to give compounds **1** (16 mg), **2** (124 mg), and **3** (5 mg).

Compound **1** was obtained as a colorless amorphous powder. The molecular formula $C_{21}H_{28}O_5$ was revealed by a high-resolution electron-impact mass spectrum (HREIMS). The close similarity of the ^{13}C NMR spectrum (Table 1) to that of an icetexane diterpene, coulterone (**4**),[3] indicated that **1** has the same 5α*H*,10β-hydroxy icetexane skeleton as **4**. Analysis of the HMQC and HMBC spectra supported this structure. However, instead of the methoxy group in **4**, the NMR spectra of **1** showed the presence of a methylenedioxy group (δ_C 100.7, δ_H 5.92 and 5.94), located at C-11,12 in the icetexane skeleton on the basis of the HMBC spectra (Table 1). Thus, **1** was concluded to be an 11,12-methylenedioxy derivative of **4** and was named cyclocoulterone.

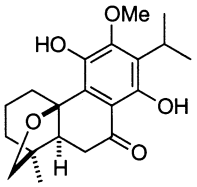
Compound **2** was obtained as an orange oil. The presence of a tetrasubstituted *p*-benzoquinone moiety was concluded from its ^{13}C NMR spectrum (δ_C 142.1 138.9, 183.6, 156.1, 137.0, 189.1), and methoxy and isopropyl groups were located on the benzoquinone ring on the basis of the HMBC spectrum (Table 1, Figure 1). Extensive analyses of the HMBC spectrum revealed that this compound also has an icetexane skeleton. On the basis of the molecular formula $C_{21}H_{28}O_5$ revealed by HREIMS, there were two remaining oxygen atoms in the molecule other than those associated with the benzoquinone moiety. One of these was due to the hydroxyl group at δ_H 5.99, which correlated with the carbon at δ_C 100.9 in the HMBC spectrum. From the chemical shift, this carbon at δ_C 100.9 should be attached to another oxygen atom, and the carbon at δ_C 79.3, located at C-10 on the basis of the HMBC spectrum, was also attached to an oxygen atom. Since there was only one oxygen atom left, these two carbons were concluded to be attached to the same oxygen atom to form a hemiketal function. The hydroxyl proton also showed an HMBC correlation with a benzoquinone carbon (δ_C 142.1), which located the hemiket-



cyclocoulterone (**1**): R_1=R_2= -O-CH_2-O-
coulterone (**4**): R_1=OMe; R_2=OH



komaroviquinone (**2**)



dracocephalone A (**3**)

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Table 1. NMR Data of **1**−**3**[a]

	4[b]	**1**			**2**			**3**		
no.	^{13}C	^{13}C	^{1}H	HMBC[c]	^{13}C	^{1}H	HMBC[c]	^{13}C	^{1}H	HMBC[c]
1	38.4	38.5	α: 1.54, dd (14, 4.3) *β*: 1.73, br d (14)		15.6	1.60, overlap	5, 10	35.5	α: 1.44, td (13.1, 5.5) *β*: 2.86, dd (13.4, 5.8)	2 2, 3, 5, 10
2	18.6	18.6	α: 1.56, dd (14, 4) *β*: 1.87, qt (13.7, 3.4)		29.8	α: 1.73, overlap *β*: 2.03, overlap	10 10	20.3	α: 1.80, dt (14.1, 5.8) *β*: 2.10, qt (13.4, 6.4)	4 1, 3, 10
3	40.6	40.6	α: 1.14, td (13.8, 2.9) *β*: 1.45, br d (13.7)	 4	31.2	α: 1.15, dd (11.3, 5.8) *β*: 1.59, overlap	1, 4, 5, 7, 19 1, 4, 18, 19	39.0	α: 1.61, tdd (12.8, 5.2, 1.3) *β*: 1.69, dd (13.1, 6.4)	19 1, 4, 5
4	33.8	34.0			32.0			43.7		
5	48.8	49.6	1.43, d (9.5)	6	51.4	1.71, t (8.2)	1, 4, 6, 18, 19	51.9	2.20, dd (11.9, 6.8)	19
6	40.5	40.0	α: 2.62, d (17.7) *β*: 3.00, dd (17.7, 9.8)	4, 5, 8, 10 10	45.7	α: 2.30, dd (12.8, 8.6) *β*: 2.04, dd (12.8, 7.8)	4, 10 4, 5, 7, 8	36.3	2.54, m	5, 6, 10
7	210.6	208.6			100.9			203.1		
8	116.7	112.5			142.1			111.7		
9	121.1	114.7			138.9			124.4		
10	73.7	73.6			79.3			83.0		
11	139.5	139.2			183.6			140.7		
12	150.1	150.6			156.1			154.1		
13	127.4	117.1			137.0			128.8		
14	157.1	160.6			189.1			155.9		
15	26.0	24.3	3.40, sep (7.0)	12, 13, 14, 15	24.3	3.23, sep (7.0)	13, 14, 15,16, 17	25.5	3.43, sep (7.0)	13, 14, 16, 17
16	20.5	20.6[d]	1.30, d (7.0)	13, 15	20.4	1.18, d (7.3)	13, 15	20.5	1.35, d (7.0)	15, 17
17	20.3	20.7[d]	1.28, d (7.4)	13, 15	20.4	1.18, d (7.0)	15	20.5	1.35, d (7.0)	13, 15, 16
18	32.0	32.0	0.86, s	3, 4, 5, 19	30.3	0.95, s	3, 4, 5, 19	18.6	1.03, s	3, 4, 5, 19
19	21.4	21.4	1.00, s	3, 4, 5, 18	27.0	0.86, s	4, 5, 18	78.1	b: 4.02, d (8.3) a: 3.89, dd (8.3, 1.3)	3 5 10 3
20	40.4	41.3	α: 3.02, d (14.3) *β*: 2.74, d (14.1)	1, 5, 8, 9, 10, 11 1, 5, 8, 9, 10, 11	39.0	α: 2.26, d (19.6) *β*: 2.55, d (19.6)	5, 7, 8, 9, 11, 14 5, 7, 8, 9, 11, 14			
OMe	61.9				61.1	3.98, s	12	61.6	3.88, s	12
OCH_2O		100.7	5.92, s and 5.94, s	11, 12						
OH[c]			1.24, br s 13.43, s	 8, 13, 14		5.99, s	7, 8		5.87, s 12.17, s	9, 11 8, 13, 14

[a] Recorded in $CDCl_3$ at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively; data in *δ* ppm (*J* in Hz). [b] Frontana, B.; Cárdenas, J.; Rodríguez-Hahn, L. *Phytochemistry* **1994**, *36*, 739−741. [c] Carbons correlated with the proton. [d] The assignments may be interchanged.

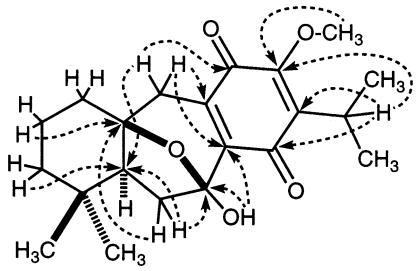

Figure 1. Key HMBC correlations in **2**.

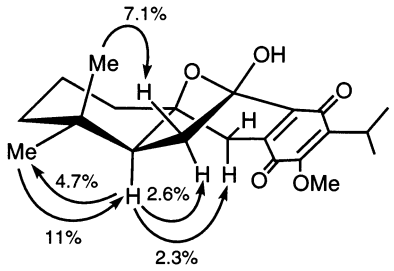

Figure 2. Selected NOEs in **2**.

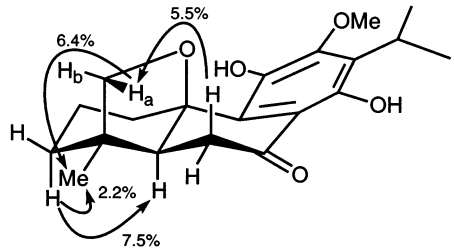

Figure 3. Selected NOEs in **3**.

al carbon at C-7 in the icetexane skeleton. In an NOE difference experiment (Figure 2), irradiation of the H-5 proton (δ_H 1.71) resulted in NOE effects on H-18 (δ_H 0.95, α methyl) and one of the H-20 protons (δ_H 2.26), indicating the *cis* relationship between 18-Me, H-5, and C-20. On the basis of these and other NOEs shown in Figure 2, the stereochemistry at C-5 and C-10 was concluded to be 5αH, 10*β*. Thus, **2** was concluded to have the structure shown and was named komaroviquinone.

Compound **3** was obtained as a colorless amorphous powder, and it showed NMR spectra similar to those of **4** (Table 1). However, the molecular formula $C_{20}H_{26}O_5$ (HREIMS) suggested the loss of a methylene unit and the formation of an extra ring in **3**. In the NMR spectra, one isolated methylene group corresponding to the C-20 of the icetexane skeleton was missing, suggesting that this was a 20-norabietane derivative. Furthermore, in place of one of the geminal methyls on the C-4 carbon in **4**, an oxymethylene group (δ_C 78.1, δ_H 3.89 and 4.02) was seen. The oxymethylene protons showed correlation peaks with C-3 (δ_C 39.0), C-5 (δ_C 51.9), and C-10 (δ_C 83.0) carbons in the HMBC spectrum, indicating the formation of an ether linkage between the oxymethylene group on the C-4 carbon and the C-10 carbon. In NOE difference experiments in benzene-d_6 (Figure 3), irradiation of the H-3α axial proton (δ_H 0.95, tdd, J = 13.1, 4.0, 1.0 Hz) resulted in NOE effects on H-18 (δ_H 0.39) and H-5 (δ_H 1.42), whereas irradiation of the H-6*β* axial proton (δ_H 1.92, t, J = 14.3 Hz) enhanced the signal intensity of the H-19a proton (δ_H 3.89). These results indicated the stereochemistry at C-5 and C-10 to be 5αH, 10*β*. Thus, **3** was determined to have the indicated structure and was named dracocephalone A.

Most of the known icetexane and 20-norabietane diterpenes have been isolated from *Salvia* species, and only a few have originated from another genus.[4−8] Thus, isolation of the above compounds from a *Dracocephalum* species is of interest from a chemotaxonomic perspective.

Komaroviquinone (**2**) showed strong trypanocidal activity against epimastigotes of *T. cruzi* with a minimum lethal concentration (MLC) of 0.4 μM. The MLC of gentian violet, which is used to disinfect trypanosomes from transfusion blood in Latin America, was 6.3 μM under the same assay condition. Several types of natural quinones have been reported to show trypanocidal activity, and their activities have been partly ascribed to the production of reactive oxygen species in the parasite.[9] The other two compounds showed moderate trypanocidal activity: **1**, MLC = 20 μM; **3**, MLC = 200 μM.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO DIP-370 polarimeter. ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM-LA500 spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given as δ values. Mass spectra were measured on a JEOL JMS-HX/HX110A spectrometer. UV and IR spectra were recorded on Hitachi U-3210 and Shimadzu FTIR-8700 spectrometers, respectively.

Extraction and Isolation. Dried whole plants of *D. komarovi* were purchased at a local market in Kumyshkan, Uzbekistan, and identified by one of the authors (O.K.K.). A voucher specimen (ESM-4235) was deposited at the Experimental Station of Medicinal Plants, Faculty of Pharmaceutical Sciences, Kyoto University.

Dried whole plants of *D. komarovi* (1.6 kg) were cut into small pieces and successively extracted with hexane and EtOAc at room temperature overnight to give hexane (22.2 g) and EtOAc (67.3 g) extracts. Each extract was subjected to silica gel column chromatography using hexane−acetone (10:1, 8:1, 6:1, 4:1, 0:1) and MeOH as eluents. The fractions eluted with hexane−EtOAc (8:1 and 6:1 from hexane extract (5.6 g) and 6:1 from EtOAc extract (4.6 g)) were fractionated by silica gel column chromatography ($CHCl_3$−acetone (100:1), benzene−EtOAc (30:1, 20:1), benzene−acetone (30:1)) and HPLC (YMC Pack SIL-06, hexane−EtOAc = 5:1) to give compounds **1** (16 mg), **2** (124 mg), and **3** (5 mg).

Cyclocoulterone (1): colorless amorphous powder, mp 142−143 °C; $[\alpha]^{25}_D$ −143.5° (*c* 0.57, MeOH); UV (MeOH) λ_{max} (log ϵ) 359 (3.83), 292 (3.88), 246 (3.84) nm; IR (KBr) ν_{max} 3479, 2928, 1643, 1597, 1435, 1408 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m*/*z* 360 [M$^+$] (22), 342 (11), 273 (12), 211 (100); HREIMS *m*/*z* 360.1950 (calcd for $C_{21}H_{28}O_5$, 360.1929).

Komaroviquinone (2): orange oil, $[\alpha]^{25}_D$ +34.2° (*c* 1.86, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 366 (2.89), 272 (3.84) nm; IR (KBr) ν_{max} 3410, 2947, 1651, 1600, 1458 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m*/*z* 360 [M$^+$] (23), 342 (8), 316 (100), 301 (30), 273 (11), 247 (20), 221 (19); HREIMS *m*/*z* 360.1934 (calcd for $C_{21}H_{28}O_5$, 360.1929).

Dracocephalone A (3): colorless amorphous powder, mp 167−168 °C; $[\alpha]^{25}_D$ +228.3° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 366 (3.71), 278 (3.86), 239 (3.90) nm; IR (KBr) ν_{max} 3464, 2932, 2870, 1636, 1420 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; ^{1}H NMR (C_6D_6, 500 MHz) δ 0.39 (3H, s, H-18), 0.96 (1H, tdd, J = 13.1, 4, 1 Hz, H-3α), 1.08 (1H, overlap, H-1α), 1.10 (1H, overlap, H-3*β*), 1.39 (1H, m, H-2α), 1.42 (1H, dd, J = 14.4, 4.3 Hz, H-5), 1.55 and 1.57 (each 3H, d, J = 7 Hz, H-16 and 17), 1.72 (1H, qt, J = 13.1, 6.7 Hz, H-2*β*), 1.92 (1H, t, J = 14.3 Hz, H-6*β*), 2.11 (1H, dd, J = 14.4, 4.4 Hz, H-6α), 2.67 (1H, dd, J = 13.4, 6.1 Hz, H-1*β*), 3.39 (1H, dd, J = 7.9, 1.5 Hz, H-19a), 3.47 (1H, d, J = 7.9 Hz, H-19b), 3.71 (3H, s, OMe), 3.82 (1H, sept, J = 7.2 Hz, H-15), 6.05 and 12.85 (each 1H, s, OH); ^{13}C NMR (C_6D_6, 125 MHz) δ 17.9 (C-18), 20.3 (C-2), 20.9 and 21.0 (C-16 and 17), 25.6 (C-15), 36.0 (C-6), 38.7 (C-3), 43.2 (C-4), 50.9 (C-5), 60.8

(OMe), 77.8 (C-19), 83.3 (C-10), 111.6 (C-8), 125.8 (C-9), 129.1 (C-13), 141.5 (C-11), 155.6 (C-12), 156.1 (C-14), 202.5 (C-7); EIMS *m*/*z* 346 [M^+] (100), 303 (22), 273 (11), 262 (18), 245 (10), 215 (10); HREIMS *m*/*z* 346.1782 (calcd for $C_{20}H_{26}O_5$, 346.1773).

Trypanocidal Assay. Trypanocidal activity against epimastigotes of *Trypanosoma cruzi* (Tulahuen strain) was determined as described previously.[10] Each assay was performed in duplicate.

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