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A trypanocidal diterpene with novel skeleton from *Dracocephalum komarovi*

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Abstract—A new diterpene, komarovispirone (**1**) with a spiro-octahydroindene skeleton, was isolated from *Dracocephalum komarovi*. The structure was elucidated by extensive analyses of spectral data. Komarovispirone (**1**) showed trypanocidal activity against epimastigote of *Trypanosoma cruzi*, the causative agent of American trypanosomiasis, with a minimum lethal concentration of 23 μM.

Dracocephalum komarovi Lipsky (Labiatae) is a perennial semishrub[1] that is called 'buzbosh' in Uzbekistan, and local people use the aerial parts in a tea to treat various diseases such as inflammatory diseases and hypertony. In a previous paper, we reported the isolation of three trypanocidal diterpenes from this plant.[2] In this paper, we report the isolation and structural determination of a new diterpene with a novel skeleton, komarovispirone (**1**).

Dried whole plants of *D. komarovi* were extracted and fractionated as described previously.[2] Separation of the fractions with strong trypanocidal activity against epimastigotes of *Trypanosoma cruzi* by silica gel column chromatography (hexane–AcOEt, $CHCl_3$–acetone, benzene–AcOEt, benzene–acetone) and HPLC (YMC Pack SIL-06, hexane–AcOEt) gave komaroviquinone (**3**) as the major trypanocidal compound.[2] Separation of a side fraction from the first silica gel column (hexane–AcOEt), which showed moderate trypanocidal activity, by silica gel column chromatography (hexane–acetone, benzene–AcOEt) and HPLC (YMC Pack SIL-06, hexane–AcOEt = 15:1) resulted in isolation of compound **1** (10 mg).[3]

Compound **1** was obtained as yellow needles from MeOH, $[\alpha]_D^{25}$ +282.6° (*c* 1.0, MeOH). The molecular formula of $C_{21}H_{28}O_5$ was revealed by high-resolution EIMS (HR-EIMS) (M^+ *m*/*z* 360.1924; calculated, 360.1937). The NMR spectra of **1** (Table 1) showed the presence of a methoxy group (δ_C 59.8, δ_H 3.71), a chelated hydroxy group (δ_H 13.32), two singlet methyls (δ_C 31.6, δ_H 0.53 and δ_C 19.8, δ_H 0.87), two methylenes [δ_C 40.3, δ_H 1.94 (1H, t, $J = 11.9$ Hz) and 1.47 (1H, dd, $J = 11.6$, 9.8 Hz); δ_C 43.6, δ_H 2.06 and 1.20, (each 1H, d, $J = 11.9$ and 12.2 Hz)] and an isopropyl group [δ_C 26.0, δ_H 3.57 (1H, sept, $J = 7.0$ Hz); δ_C 20.6, δ_H 1.37 (3H, d, $J = 7.1$ Hz); δ_C 20.4, δ_H 1.36 (3H, d, $J = 7.0$ Hz)]. These spectra were similar to those of the icetexane diterpene, komaroviquinone (**3**),[2] and the HMBC spectrum revealed that the hydroxy, methoxy and isopropyl groups were in the same arrangement as in **3** (Fig. 1). However, protons of the methylenes corresponding to C-6 (δ_C 40.3) and C-20 (δ_C 43.6) in **1** showed correlation peaks with the carbonyl carbon at δ_C 195.5, the oxygenated quaternary carbon at δ_C 91.5, and a quaternary carbon at δ_C 51.7, indicating the skeleton to be different from that of icetexane in the C-7 to C-9 part. The protons of the two methylenes also showed correlations with the olefinic carbon (δ_C 107.1), which was correlated to the

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Table 1. NMR data of **1** in C_6D_6[a]

No.	^{13}C	^{1}H	HMBC[b]
1	34.4	α 0.89, td, 13.1, 4.6	2
		β 1.75, br d, 13.1	10
2	18.5	α 1.25, m, overlap	
		β 1.89, qt, 13.7, 3.4	
3	40.4	α 0.75, td, 13.7, 4.0	19
		β 1.25, m, overlap	1, 5
4	33.5	—	
5	55.4	1.39, dd, 11.0, 9.4	4, 6, 19
6	40.3	α 1.94, t, 11.9	5, 8, 9, 10, 11
		β 1.47, dd, 11.6, 9.8	5, 8, 9, 20
7	169.6	—	—
8	107.1	—	—
9	51.7	—	—
10	91.5	—	—
11	195.5	—	—
12	154.7	—	—
13	143.6	—	—
14	160.4	—	—
15	26.0	3.57, sept, 7.0	12, 13, 14, 16, 17
16	20.6[c]	1.37, d, 7.1 (3H)	13, 15, 17
17	20.4[c]	1.36, d, 7.0 (3H)	13, 15, 16
18	31.6	0.53, s (3H)	3, 4, 5, 19
19	19.8	0.87, s (3H)	3, 4, 5, 18
20	43.6	2.06, d, 11.9	8, 9, 11
		1.20, d, 12.2	5, 6, 8, 10
OMe	59.8	3.71, s (3H)	12
OH		13.32, s	8, 13, 14

[a] ^{1}H NMR, 500 MHz; ^{13}C NMR, 125 MHz; data in δ ppm (J in Hz).
[b] Carbons correlated with the proton.
[c] The assignments may be interchanged.

chelated hydroxy proton. From these data and other HMBC correlations, a partial structure as shown in Figure 2 was established. Since one carbon and one oxygen atoms should be added to this partial structure to satisfy its molecular formula $C_{21}H_{28}O_5$, a carbonyl group (δ_C 169.6) was connected to X and Y of the partial structure (Fig. 2) to form a lactone. Thus, the structure of **1** was concluded as indicated, and since it has a novel spiro-octahydroindene skeleton, it is named komarovispirone.

This structure was supported by a chemical conversion. On heating in 5% HCl/MeOH, **1** gave compound **2**.[4] From HR-EIMS (M^+ m/z 302.1886, calculated, 302.1882), the molecular formula of **2** was revealed to be $C_{19}H_{26}O_3$, which corresponds to simultaneous occurrence of decarboxylation and demethylation. The NMR spectra (Table 2) showed the presence of a hydroxy group (δ_H 7.17), three isolated methylenes (δ_C 42.5, δ_H 2.90 and 2.13; δ_C 47.4, δ_H 2.85 and 2.07; δ_C 50.3, δ_H 2.77 and 2.74), and two ketone carbonyls (δ_C 196.6 and 198.8), but no signal of the methoxy and lactone groups was observed. Based on the HMBC correlations (Table 2), the structure of **2** was concluded as indicated, and this structure is fully compatible with the structure of **1**.

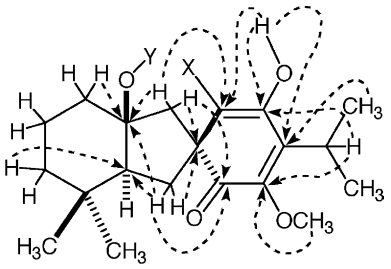


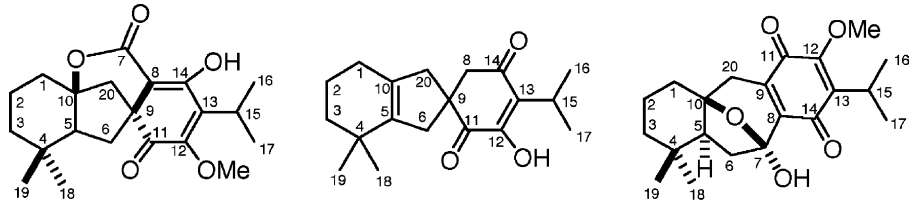
Figure 2. Selected HMBC correlations and partial structure of **1**.

Table 2. NMR data of **2** in $CDCl_3$[a]

No.	^{13}C	^{1}H	HMBC[b]
1	25.8	1.84, br s (2H)	2, 3, 5, 10
2	19.7	1.62, m (2H)	1, 3, 4, 10
3	38.5	1.40, m (2H)	1, 18, 19
4	32.1	—	—
5	138.9	—	—
6[c]	47.4	2.85, d, 15.8	5, 8, 9, 10
		2.07, d, 15.8	5, 8, 9, 10
8	50.3	2.77, d, 15.5	6, 9, 11, 14, 20
		2.74, d, 15.5	6, 9, 11, 14, 20
9	50.1	—	—
10	130.2	—	—
11	198.8	—	—
12	153.5	—	—
13	131.5	—	—
14	196.6	—	—
15	24.7	3.18, sept, 7.2	12, 13, 14, 16, 17
16	19.5	1.20[c], d, 7.2 (3H)	13, 15, 17
17	19.5	1.21[c], d, 6.9 (3H)	13, 15, 16
18,19	27.5	0.94, s (6H)	3, 4, 5, 18, 19
20[c]	42.5	2.90, br d, 15.8	
		2.13, dd, 15.8, 2.1	5, 9, 10
OH		7.17, s	11, 12, 13

[a] ^{1}H NMR, 600 MHz; ^{13}C NMR, 150 MHz; data in δ ppm (J in Hz).
[b] Carbons correlated with the proton.
[c] The assignments may be interchanged.

NOE difference experiments in $CDCl_3$ and/or benzene-d_6 showed NOE effects indicated in Figure 3. Although these results suggested the stereochemistry shown in Figure 3, the entire stereochemistry could not be determined. Biogenetically, komarovispirone (**1**) may be



komarovispirone (**1**) **2** komaroviquinone (**3**)

Figure 1.

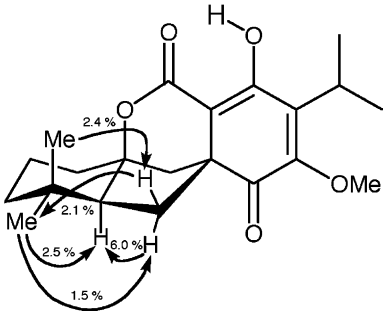
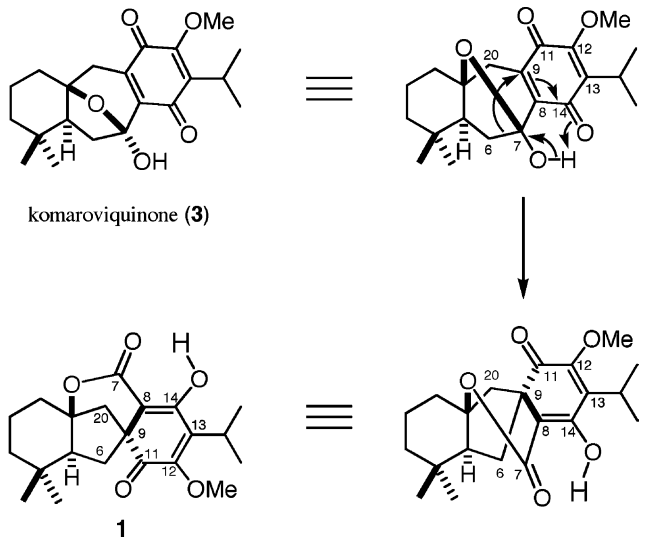


Figure 3. Selected NOEs in **1**.



Scheme 1. Possible route of formation of **1**.

derived from komaroviquinone (**3**), which is isolated from the same plant,[2] through a rearrangement shown in Scheme 1. Thus, the stereochemistry of **1** was tentatively assigned as indicated.

Komarovispirone (**1**) showed moderate trypanocidal activity against epimastigote of *T. cruzi*[5] with a minimum lethal concentration (MLC) of 23 μM. The MLC of **1** was higher than that of **3** (0.4 μM) under the same assay condition.[2] Several types of natural quinones have been reported to show trypanocidal activity, and their activities have been partly ascribed to production of a reactive oxygen species in the parasite.[6,7] Thus, the action mechanism of **1** may be similar to that of these quinone compounds.

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3. Komarovispirone (**1**): mp 119–120 °C; UV λ_{max}^{MeOH} nm (log ε): 251 (4.52), 365 (3.06); IR (KBr) cm^{-1}: 2943, 2874, 1651, 1558; EI-MS *m/z* (%): 360 (M^+, 26), 316 (100), 301 (61), 283 (17). ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in C_6D_6: see Table 1.
4. Komarovispirone **1** (1 mg) was heated in 5% HCl/MeOH (0.5 mL) at 65 °C for 3.5 h. The reaction mixture was concentrated to dryness to give compound **2** (0.6 mg). Compound **2**: colorless amorphous powder, mp > 300 °C; UV λ_{max}^{MeOH} nm (log ε): 288 (3.95), 212 (3.83); IR (KBr) cm^{-1}: 3360, 2928, 2870, 1666; EI-MS *m/z* (%): 302 (M^+, 100), 287 (88). ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) in $CDCl_3$: see Table 2.
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