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MOLECULAR STRUCTURE AND ABSOLUTE CONFIGURATION OF TATRIDIN A AND B FROM *Lepidolopha karatavica*

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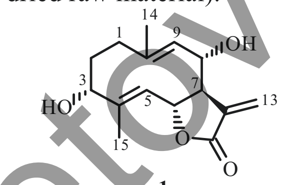
The germacrane-type sesquiterpene lactones tatridin A and B were isolated for the first time during chemical studies of the extract of Lepidolopha karatavica*. The structures of the compounds were elucidated using spectral data and X-ray crystal structure analyses (XSAs). Correlations of the XSAs and CD spectra defined the absolute configurations of the molecules. Possible conformational states of 3,8-deoxytatridin A and B were calculated by a quantum chemical method.*

Keywords: sesquiterpene lactones, *Lepidolopha karatavica*, crystal structure, NMR, XSA, molecular conformation.

Plants of the genus *Lepidolopha* Winkl. (Asteraceae) are represented by six species in the CIS, of which three inhabit Kazakhstan [1]. The chemical composition of *L. karatavica* Pavlov, an endemic plant species collected on May 28–30, 2010, during flowering on rocky slopes of Syrdariya Karatau, South-Kazakhstan Region, was studied by us.

Finely ground leaves of *L. karatavica* (2 kg) were extracted (3×) with $CHCl_3$ with heating. The dried extract after workup with aqueous EtOH afforded total extracted substances (86.81 g), from which column chromatography using petroleum ether–EtOAc (85:15) isolated colorless crystalline **1**, mp 170–173°C. The yield of **1** was 0.519 g (0.026% of the air-dried raw material). Subsequent elution of the column by this same mixture isolated colorless crystalline **2**, mp 172–175°C, yield 0.359 g (0.018% of the air-dried raw material).



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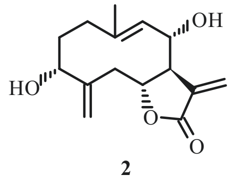

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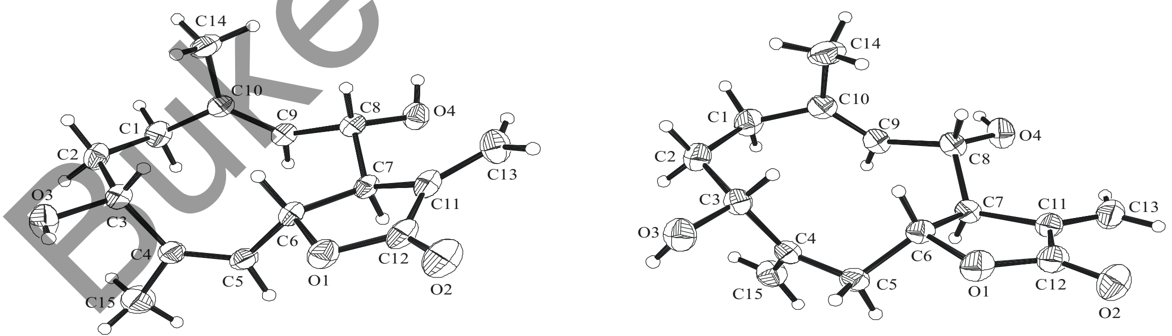

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Fig. 1. Molecular structures of tatridin A (**1**) and B (**2**) (30% probability thermal ellipsoids are shown).

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TABLE 1. PMR (600 MHz) and ^{13}C NMR Data (150 MHz) for **1** and **2** ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1a	2.28 (q, J = 6.0)	35.83 (CH_2)	2.26 (m)	34.72 (CH_2)
1b	1.92 (m)		2.06 (m, overl.)	
2a	1.98 (m)	27.87 (CH_2)	2.06 (m, overl.)	30.94 (CH_2)
2b	1.74 (m)	–	2.06 (m, overl.)	
3	4.36 (dd, J = 5.0, 10.5)	66.81 (CH)	3.83 (t, J = 7.0)	70.20 (CH)
4	–	143.43 (C)	–	146.67 (C)
5	5.31 (d, J = 9.0)	126.93 (CH)	2.40 (dd, J = 10.0, 14.0)	42.03 (CH_2)
6	4.65 (t, J = 9.0)	75.89 (CH)	4.01 (br.s)	80.27 (CH)
7	2.80 (m)	52.94 (CH)	2.82 (m)	52.42 (CH)
8	4.45 (dd, J = 9.0, 10.0)	70.95 (CH)	4.21 (t, J = 10.0)	70.27 (CH)
9	4.96 (d, J = 10.0)	131.10 (CH)	5.05 (d, J = 10.0)	131.88 (CH)
10	–	134.37 (C)	–	134.00 (C)
11	–	139.00 (C)	–	137.51 (C)
12	–	171.91 (C)	–	171.69 (C)
13a	6.24 (d, J = 2.0)	123.53 (CH_2)	6.30 (d, J = 2.5)	125.87 (CH_2)
13b	6.23 (d, J = 2.0)	–	6.23 (d, J = 2.0)	
14	1.79 (3H, s)	15.54 (CH_3)	1.71 (3H, s)	17.35 (CH_3)
15	1.82 (3H, s)	17.02 (CH_3)	5.15 (s); 5.11 (s)	114.85 (CH_2)

TABLE 2. Intracyclic Torsion Angles (τ, deg) in the Structures of **1** and **2**

Angle	**1a**	**1b**	**1c**	**1d**	**2**
C10-C1-C2-C3	–46.2(6)	–43.9(6)	–45.1(7)	–45.8(6)	–32.2(2)
C1-C2-C3-C4	–51.9(6)	–54.4(6)	–52.6(6)	–52.0(6)	–58.1(2)
C2-C3-C4-C5	117.4(6)	115.5(5)	116.7(5)	120.5(5)	155.5(2)
C3-C4-C5-C6	–2.1(9)	0.0(8)	–2.3(8)	–3.3(8)	–66.0(2)
C4-C5-C6-C7	–121.5(6)	–126.5(5)	–125.7(5)	–122.9(5)	–73.7(2)
C5-C6-C7-C8	91.3(5)	91.9(5)	92.7(5)	90.6(5)	109.6(2)
C6-C7-C8-C9	–48.5(5)	–45.5(5)	–47.3(5)	–48.0(5)	–64.9(2)
C7-C8-C9-C10	112.9(5)	109.4(5)	108.9(5)	113.4(5)	117.7(2)
C8-C9-C10-C1	–162.0(5)	–162.4(4)	–161.4(4)	–161.0(5)	–166.2(2)
C2-C1-C10-C9	112.1(5)	113.0(5)	114.5(5)	110.4(5)	102.1(2)
O1-C6-C7-C11	–25.3(4)	–25.0(5)	–23.2(5)	–25.3(4)	–7.8(2)
C6-C7-C11-C12	22.1(4)	20.8(5)	18.6(5)	20.3(4)	5.6(2)
C7-C11-C12-O1	–10.8(5)	–8.7(6)	–6.8(7)	–7.5(5)	–1.1(2)
C6-O1-C12-C11	–6.8(5)	–8.5(6)	–9.0(6)	–9.8(5)	–4.3(2)
C12-O1-C6-C7	20.8(5)	21.6(5)	20.7(6)	22.7(5)	7.8(2)

The isolated compounds were identified as tatridin A (**1**) and tatridin B (**2**), which were isolated earlier from *Artemisia tridentata* [2], according to PMR and ^{13}C NMR spectra, two-dimensional NMR ^{1}H–^{1}H (COSY, NOESY) and ^{13}C–^{1}H spectra (HMQC, COLOC), and X-ray crystal structure analyses (XSAs). Table 1 presents the NMR data and assignment of resonances for **1** and **2**.

Figure 1 shows general views of the crystal hydrate of **1** and compound **2** from the XSAs.

The XSA data showed that the bond lengths and angles in **1** and **2** were close to normal [3]. The 10-membered carbocycle in **1** and **2** had a nonstandard C9=C10 double bond, which is usually formed between C1–C10 [4]. Nevertheless, the 10-membered ring in **1** could be arbitrarily assigned the $_{15}D_{5},_{9}D^{14}$ conformation according to Samek and Harmatha [5]. The crystal cell of **1** contained four independent molecules (**1a**–**1d**) that adopted the same conformation but with a slight scatter in the torsion angles [Δτ(max) = 5.0°] (Table 2). The 10-membered ring of the only other tatridin A (diacetate) studied by XSA had a similar conformation [6].

The conformation of the C3…C6 portion of the 10-membered ring of **2** differed significantly from that of **1**. The torsion angles observed for the C3–C4, C4–C5, and C5–C6 bonds differed because of absence the C4=C5 double bond in **2** (Table 2).

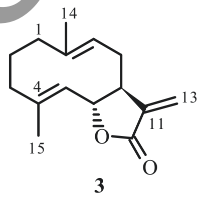
TABLE 3. Intracyclic Torsion Angles (τ, deg), Heats of Formation (ΔH_f, kJ/mol), and Total Energy (E_{tot}, kJ/mol) in Conformers of **3** and **4**

Angle	**3a**	**3b**	**4a**	**4b**
C10-C1-C2-C3	–35.5	–140.0	–92.3	–68.4
C1-C2-C3-C4	–62.6	58.8	70.9	70.9
C2-C3-C4-C5	123.2	41.0	64.0	–124.5
C3-C4-C5-C6	–5.5	–3.0	–99.3	162.1
C4-C5-C6-C7	–121.5	–115.1	–60.3	–92.4
C5-C6-C7-C8	99.6	125.4	115.6	–61.0
C6-C7-C8-C9	–52.2	–44.4	–51.8	–50.6
C7-C8-C9-C10	112.4	–72.4	100.7	133.9
C8-C9-C10-C1	–165.1	121.0	–159.0	–167.8
C2-C1-C10-C9	108.8	13.4	89.7	102.5
O1-C6-C7-C11	–17.5	4.9	–5.0	30.0
C6-C7-C11-C12	15.4	–3.1	4.6	–25.2
C7-C11-C12-O1	–7.7	0.4	–2.5	–10.8
C6-O1-C12-C11	–4.1	3.0	–0.9	9.5
C12-O1-C6-C7	13.7	–4.9	3.7	–25.0
ΔH_f	–293.38	–301.52	–282.18	–265.13
E_{tot}	–262162.25	–262170.04	–262150.56	–262133.03

This same conformation of a 10-membered ring was observed in the crystal structure of dihydrochrysanolide [7]. Another conformation of this ring that differed in the torsion angles in the more labile C1…C6 part was observed in the crystal structure of tatridin B 4,8-dibenzoate [8].

The lactone ring in **1** adopted a distorted 6β,7α-half chair conformation ($\Delta C_2^{6,7}$ = 3.0, 0.6, 2.2, and 2.4° for **1a**–**1d**, respectively). It was an almost ideal but strongly flattened 7α-envelope (ΔC_S^{7} = 0.9°) in **2**. The lactone and carbocycle were *trans*-fused [torsion angles H6C6C7H7 = –154 (**1a**), –154 (**1b**), –152 (**1c**), and –154 (**1d**) and –135°C (**2**)]. The C3 and C8 hydroxyls had equatorial orientations. Tatridin A and B were assigned the structures 3α,8α-dihydroxy-6β,7α(*H*)-germacr-4,9,11(13)-trien-6,12-olide and 3α,8α-dihydroxy-6β,7α(*H*)-germacr-4(15),9,11(13)-trien-6,12-olide based on the XSAs.

According to the Samek classification, the conformation of the 10-membered 4,1(10)-diene carbocycle could be characterized by four states with different α- and β-orientations of the C4 and C10 methyls [5]. By analogy, the four conformational states with different α- and β-orientations of the C4 and C10 methyls was modeled by us for the hypothetical molecule 3,8-deoxytatridin A (**3**), which contained the basic germacranolide skeleton. The geometries of the modeled possible conformers of **3** were optimized by a semi-empirical quantum chemical method to find their stabilities. For this, the conformational states of the model molecule 3,8-deoxytatridin B (**4**) were calculated using data from the literature.



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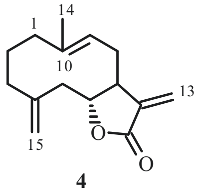

4

The quantum-chemical calculations of the geometric and energetic parameters of **3** established that two of the four possible conformers were stable, i.e., **3a**, with the ${}_{15}D_{5},{}_{9}D^{14}$ conformation of the 10-membered ring, and **3b**, with the ${}_{15}D_{5},{}_{9}D_{14}$ conformation (Table 3). The energetic characteristics of conformer **3b** were more favorable than those of conformer **3a** by 8 kJ/mol.

Quantum-chemical calculations were performed for **4** in the two conformational states of the 10-membered ring that occurred in tatridin B (**4a**) and tatridin B 4,8-dibenzoate (**4b**). Optimization of the geometry of the conformers found that conformer **4a** was significantly more stable by ~17 kJ/mol than that of conformer **4b** (Table 3). The torsion angles in the C1…C4 portion of **4a** differed significantly from those in **2**.

TABLE 4. Crystallographic Data and Characteristics of X-ray Crystal Structure Analyses of the Crystal Hydrate of **1** and Compound **2**

Parameter	**1**	**2**
Empirical formula	$C_{15}H_{20}O_4 \cdot H_2O$	$C_{15}H_{20}O_4$
M	282.32	264.31
System	Trigonal	Orthorhombic
T, K	299	299
a, Å	15.8506(5)	8.6223(3)
b, Å	15.8506(5)	10.6929(5)
c, Å	22.0543(9)	15.3566(6)
γ, deg	120	90
V, $Å^3$; *Z*	4798.6(4); 12	1415.8(1); 4
Space group	$P3_1$	$P2_12_12_1$
D_{calcd}, g/cm^3	1.172	1.240
μ, mm^{-1}	0.087	0.089
Number of measured reflections	198391	13509
Number of independent reflections	15933 (R_{int} = 0.0491)	3304 (R_{int} = 0.0185)
Number of observed reflections ($I \geq 2\sigma(I)$)	13008	3004
Number of refined parameters	703	182
F(000)	1824	568
Range of θ, deg	$2.37 \leq \theta \leq 28.36$	$2.65 \leq \theta \leq 27.72$
R_1, wR_2 (all data)	0.0753, 0.2170	0.0325, 0.0863
R_1, $_{W}R_2$ (whole array)	0.0910, 0.2309	0.0370, 0.0895
GooF	1.062	1.029
$\Delta\rho_{max}$, $\Delta\rho_{min}$, e/$Å^3$	0.988, –0.322	0.141, –0.128

The absolute configuration of the chiral centers in **1** and **2** were determined from the sign of the Cotton effect (CE) for the n→π* transition of the C=C–C=O chromophore. A negative CE was observed in both molecules with a maximum at 238 nm (molar circular dichroism ε = –0.15) for **1** and at 258 nm (ε = –0.16) for **2**. The absolute configurations of the molecules were established based on the correlation of the sign of the CE and the torsion angles C13C11C12O2 = –12.3(9), –12.4(11), –8.0(12), and –6.5(9)° for **1** and –1.6(3)° for **2** [9, 10].

Thus, the two germacrane-type sesquiterpene lactones tatridin A and B were isolated for the first time from *L. karatavica*. The absolute configurations 3*R*,8*S*-dihydroxy-6*R*,7*R*-germacr-4,9,11(13)-trien-6,12-olide and 3*R*,8*S*-dihydroxy-6*R*,7*R*-germacr-4(15),9,11(13)-trien-6,12-olide were established for tatridin A and B based on the correlation of CD spectra and the XSAs.

EXPERIMENTAL

Melting points were determined on a Boetius apparatus. CD spectra were recorded on a Jasco-600 spectropolarimeter. NMR spectra were taken on a Bruker AV-600 spectrometer. Column chromatography used KSK silica gel and mixtures of petroleum ether and EtOAc of increasing content of the latter (from 0 to 100%).

Extraction. Finely ground leaves (2 kg) of *L. karatavica* were extracted (3×) with $CHCl_3$ with heating for 2 h and a raw-material to extractant ratio of 1:10. The $CHCl_3$ extracts were combined and evaporated in a rotary evaporator. The resulting extract (153.5 g) was worked up (3×) with EtOH–H_2O (2:1) at 70–75°C. Precipitated ballast substances were separated by decantation. The solution was filtered. The filtrate was extracted with $CHCl_3$ (4 × 0.5 L). The $CHCl_3$ extracts were combined and evaporated to dryness.

Total extracted substances (86.8 g) were mixed with adsorbent (1736 g, KSK silica gel, 0.5 mm fraction) and transferred to a chromatography column with a total to sorbent ratio of 1:20. The column was eluted by petroleum ether and petroleum ether–EtOAc mixtures with increasing content of the latter (from 0 to 100%). Elution of the column by petroleum ether–EtOAc (85:15) isolated **1** as a colorless crystalline compound, mp 170–173°C, $C_{15}H_{20}O_4$, yield 0.519 g (0.026% recalculated for air-dried raw material). Table 1 lists the PMR and ^{13}C NMR spectra.

Further elution by this same mixture afforded **2** as a colorless crystalline compound, mp 172–175°C, $C_{15}H_{20}O_4$, yield 0.359 g (0.018% recalculated for air-dried raw material). Table 1 lists the PMR and ^{13}C NMR spectra.

The calculations used the semi-empirical quantum-chemistry method with PM6 parametrization in the MOPAC 2009 software suite [11].

XSAs of 1 and 2. Cell constants and intensities of reflections were measured on a Bruker Kappa APEX2 CCD diffractometer (Mo $K\alpha$; graphite monochromator; ω,φ- and ω-scanning for the crystal hydrate of **1** and compound **2**, respectively). The initial dataset was processed and absorption was considered using the SAINT and SADABS programs (multi-scan, $T_{min} = 0.951$ and $T_{max} = 0.990$ for crystal hydrate **1**; $T_{min} = 0.956$ and $T_{max} = 0.985$ for **2**) embedded in the APEX2 software [12].

The structures were solved by direct methods. Positions of nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods. H atoms of hydroxyls in **1** and **2** were found in difference syntheses and refined isotropically in **2** and isotropically in **1** with fixed positional and thermal parameters. H atoms of the water of hydration could not be located. The remaining H atoms and their positions were also refined isotropically with fixed positional and thermal parameters (rider model).

The structures were solved and refined using SHELXS-97 [13] and SHELXL-2018/3 [14] programs. Table 4 lists the main crystallographic data and characteristics of the XSAs. The XSA data were deposited as CIF files in the Cambridge Crystallographic Data Centre (CCDC 1997093 for **1** and 1997076 for **2**).

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