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STRUCTURE AND ANTIARHYTHMIC ACTIVITY OF 12-ACETYL-12-EPINAPELLINE, A NEW DITERPENOID ALKALOID FROM *Aconitum soongoricum*

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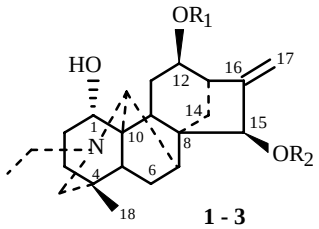
The structure of the new diterpenoid alkaloid 12-acetyl-12-epinapelline that was isolated from the mother liquor of Aconitum soongoricum *was established. The conformational state of ring A was analyzed. Spectral properties and data for the antiarhythmic activity were presented.*

Key words: diterpenoid alkaloid, 12-acetyl-12-epinapelline, x-ray structure analysis, antiarhythmic activity.

In continuation of research on alkaloids from *Aconitum soongoricum* Stapf., we isolated from the crude mother liquor of aconitine the previously observed songorine and napelline [1], karacoline [2], and base **1**, mp 198.5-200.5°C (acetone). Base **1** has an IR spectrum that shows esters (1729, 1233, 1031 cm^{-1}), hydroxyls (3430 cm^{-1}), and terminal methylene (3069, 1656, 878 cm^{-1}). The presence of a terminal methylene in **1** was confirmed by PMR spectra of the alkaloid. Broad 1H doublets with J = 2.0 Hz are observed at δ 4.88 and 5.12. The PMR spectrum indicates that **1** contains also a tertiary methyl [δ 0.71, s, 3H, C(18)–3H], N-ethyl (δ 0.98, t, J = 7.0 Hz, 3H, N–CH_2–CH_3), and acetyl (δ 1.9, s, 3H). The PMR spectrum of **1** has 1H signals at (δ, J/Hz) 2.94 (dd, J_1 = 9.0 and J_2 = 4.0, H-13), 3.23 (br.s, H-20), 3.83 (br.t, J = 7.0, H-1β), 4.14 (br.d, J = 9.0, H-15α).

The IR spectrum and PMR spectrum of **1** are similar to those for 12-epinapelline (**2**) [3] and indicate that the base isolated by us is the monoacetyl derivative of 12-epinapelline. This is confirmed by the mass spectrum, in which a peak for the molecular ion is found at *m*/*z* 401.

The literature [4] describes 12-epi-lucidusculine (**3**), a monoacetyl derivative of 12-epinapelline, the PMR spectrum of which indicates that **1** and **3** are one and the same compound. The lack of a specimen of **3** and certain difficulties in assigning the signals from H-12α and H-15α in the spectrum of **1** prompted us to continue the work on elucidating the structure of this alkaloid.



1 - 3

1: R_1 = $COCH_3$, R_2 = H; **2:** R_1 = R_2 = H; **3:** R_1 = H, R_2 = $COCH_3$

The complete structure of the isolated compound was proved by an x-ray structure analysis (XSA). The results of a single-crystal XSA of **1** showed that it is new and has the structure 12-acetyl-12-epinapelline.

The diterpenoid alkaloid **1** contains a perhydrophenanthrene core and the following placement of functional groups: an OH at the C1 α-position, an OAc at C12, and a OH at the C15 β-position. The fusion of rings is typical of the napelline framework and is retained.

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TABLE 1. Bond Lengths (r, Å) and Angles (ω, deg) in **1**

Bond	r	Angle	ω	Angle	ω
N-C21	1.472 (7)	C21-N-C19	109.5 (5)	C10-C9-C8	103.7 (4)
N-C19	1.473 (7)	C21-N-C20	109.9 (4)	C1-C10-C20	118.4 (4)
N-C20	1.479 (6)	C19-N-C20	116.8 (4)	C1-C10-C5	112.7 (4)
O1-C1	1.437 (6)	C23-O2-C12	116.5 (5)	C20-C10-C5	98.1 (4)
O2-C23	1.344 (7)	O1-C1-C2	108.0 (4)	C1-C10-C9	114.8 (4)
O2-C12	1.448 (6)	O1-C1-C10	113.3 (4)	C20-C10-C9	102.5 (4)
O3-C23	1.188 (9)	C2-C1-C10	114.9 (4)	C5-C10-C9	108.5 (4)
O4-C15	1.l433 (7)	C3-C2-C1	112.4 (5)	C9-C11-C12	115.3 (4)
C1-C2	1.530 (7)	C2-C3-C4	112.1 (4)	O2-C12-C11	107.9 (4)
C1-C10	1.534 (7)	C3-C4-C18	108.0 (5)	O2-C12-C13	112.3 (4)
C2-C3	1.515 (9)	C3-C4-C19	112.0 (5)	C11-C12-C13	112.7 (4)
C3-C4	1.521 (9)	C18-C4-C19	107.7 (5)	C16-C13-C14	99.9 (5)
C4-C18	1.533 (8)	C3-C4-C5	109.1 (5)	C16-C13-C12	114.8 (4)
C4-C19	1.551 (8)	C18-C4-C5	111.8 (5)	C14-C13-C12	108.1 (4)
C4-C5	1.546 (7)	C19-C4-C5	108.3 (4)	C8-C14-C13	101.2 (4)
C5-C10	1.559 (7)	C4-C5-C10	110.0 (4)	O4-C15-C16	113.0 (4)
C5-C6	1.562 (8)	C4-C5-C6	110.4 (5)	O4-C15-C8	116.5 (4)
C6-C7	1.536 (8)	C10-C5-C6	103.0 (4)	C16-C15-C8	104.0 (4)
C7-C8	1.526 (7)	C7-C6-C5	103.7 (4)	C17-C16-C13	125.6 (6)
C7-C20	1.574 (7)	C8-C7-C6	109.0 (4)	C17-C16-C15	126.0 (5)
C8-C14	1.533 (7)	C8-C7-C20	100.9 (4)	C13-C16-C15	108.3 (4)
C8-C15	1.538 (7)	C6-C7-C20	101.8 (4)	N-C19-C4	116.2 (4)
C8-C9	1.582 (7)	C7-C8-C14	115.2 (4)	N-C20-C10	114.0 (4)
C9-C11	1.519 (7)	C7-C8-C15	116.8 (4)	N-C20-C7	118.8 (4)
C9-C10	1.563 (6)	C14-C8-C15	99.0 (4)	C10-C20-C7	94.6 (4)
C10-C20	1.543 (6)	C7-C8-C9	102.4 (4)	N-C21-C22	113.2 (5)
C11-C12	1.525 (7)	C14-C8-C9	110.6 (4)	O3-C23-O2	123.2 (6)
C12-C13	1.544 (8)	C15-C8-C9	113.3 (4)	O3-C23-C24	125.5 (6)
C13-C16	1.515 (8)	C11-C9-C10	115.9 (4)	O2-C23-C24	111.1 (6)
C13-C14	1.547 (8)	C11-C9-C8	110.9 (4)		
C15-C16	1.519 (8)				
C16-C17	1.314 (8)				
C21-C22	1.513 (8)				
C23-C24	1.500 (11)				

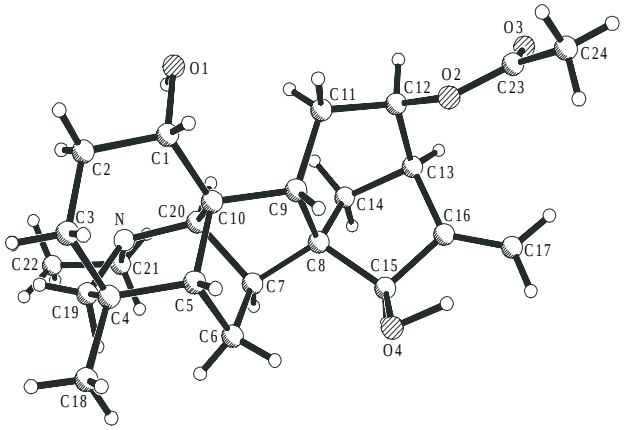

Fig. 1. Molecular structure and atomic numbering in **1**.

Figure 1 illustrates the molecular structure of **1**. The carbon skeleton is a rigid three-dimensional framework consisting of three six-membered (A, B, C) and a five-membered (D) ring and a heterocycle (E). Furthermore, the bridging bond C7–C20 forms an additional five-membered ring. The fusion of the main rings is A/B-*trans* and B/C-*cis*.

The six-membered ring A adopts an ideal chair conformation (four atoms deviate from a plane by less than ±0.003 Å); rings B (± 0.007 Å) and C (±0.03 Å), boats. Ring D has the envelope conformation (±0.05 Å) with C14 deviating from the remaining four ring atoms by 0.76 Å. Heterocycle E also has the chair conformation but is slightly distorted (flattened toward the heteroatom).

Ring A is considered flexible within the rigid carbon framework of napelline because the α-OH at C1 can either form an intramolecular H-bond with the N heteroatom or participate in intermolecular H-bonds. Depending on which H-bond predominates, ring A can adopt the boat or chair conformation, respectively. However, other canonical forms can also form. This can be seen from the following examples. In lucidusculine hydroiodide, which is known from the x-ray structure to be the 12-epimer of **3** [5], ring A has the boat conformation (intramolecular H-bond, N...O 2.88 Å) whereas in acophine [6], ring A has the twist-chair conformation (weak intramolecular H-bond, 3.14 Å). In **1**, ring A adopts the ideal chair conformation (no intramolecular H-bond, 3.64 Å).

An investigation of the structures of diterpenoid alkaloids with the lycoctonine carbon skeleton revealed [7] that if the crystals are obtained as salts, then ring A with a OH in the C1 α-position always adopts the boat conformation. The examples given above indicate that this rule extends to alkaloids with the napelline carbon framework.

Table 1 lists the bond lengths and angles. An analysis of the bond lengths indicates that the Csp^3–Csp^3 single bond lengths vary over a wide range, 1.515-1.582 Å, although the C–N heterobond and the hydroxyl are stable in the ranges 1.472-1.479 Å and 1.433-1.437 Å, respectively. The double-bond lengths (C16–C17) and bonds to the OAc are also close to the statistically average values [8].

The packing in **1** shows that the molecules in the crystal form intermolecular H-bonds of the O–H...O and O–H...N types. However, the latter is rather weak (O4...N, 3.085; O4–H...N, 2.496 Å; O4–H...N, 117.4°). The OH in the C1 position of the asymmetric unit forms a H-bond with the OH in the C15 position of the molecule translated along the 2_1 screw axis: O1...O4 Å, 2.881; O1–H...O4, 2.030 Å, angle O1–H...O4, 158.6°. As a result, an infinite chain directed along the *b* axis is formed.

Pharmacologic Investigations. Intravenous administration of **1** to mice at doses of 25-60 mg/kg led to the development of a complex of symptoms characteristic of central-nervous-system (CNS) stimulation: exhanced motor activity, tactile and audiogenic hyperreflexivity, group toxicity, tremors, cramps. Animals began to perish from toxic doses during the first two hours after administration from pulmonary and cardiovascular failure. The LD_{50} for i.v. administration of **1** to mice was 40.5 mg/kg.

In experiments with anesthetized mice, i.v. administration of **1** at doses of 10-20 mg/kg led to characteristic class-1 quinidine-like antiarhythmia changes in the EKG: brachycardia; an increase in the intervals R—R, PQ, QT; a decrease in the amplitude of the R peak, a broadened P peak, and a deepened S peak.

In the model of cardiac arhythmia elicited in rats by aconitine, **1** at doses of 5-20 mg/kg had a protective and diminishing antiarhythmic effect (Fig. 2).

According to the antiarhythmic activity and index (AAI), **1** exceeded quinidine and novocainamide: ED_{50} for **1**, 6.1; quinidine, 15.4; novocainamide, 40.7 (Table 2).

It was found earlier that songorine, napelline, 12-epinapelline (**2**), and karacoline have distinct antiarhythmic activity [9]. For comparison, Table 2 includes data for the toxicity and antiarhythmic effectiveness of napelline, **2**, and **1**. An analysis of napelline and **2** indicates that the configuration of the C12 OH has no substantial effect on the toxicity and antiarhythmic activity (within experimental uncertainty, $P \geq 0.05$). Introduction of the acetyl at the 12-position in **2** doubles the toxicity of **1** whereas the antiarhythmic activity increases by only 1.3 times. As a result, the cardioselectivity of the compound decreases. The antiarhythmic index for **2** was 10.3; for **1**, 6.6. Simultaneously with the change of toxicity, antiarhythmic activity, and selectivity of the compared compounds, the nature of the resorption also changes, i.e., the direction of the pharmacologic effect. If the nature of the resorption is compared in animals after administration of the compared compounds, symptoms characteristic of CNS stimulation increase in the order napelline $\rightarrow$ **2** $\rightarrow$ **1**.

Thus, **2** possesses antiarhythmic and CNS-stimulating activities. Introduction of an acetyl in the 12-epi-position increases the toxicity and the CNS-stimulating effect and decreases the cardioselectivity.

TABLE 2. Toxicity and Antiarhythmic Activity of Compared Compounds in the Rat Aconitine Arhythmia Model

Compound	LD_{50}, mg/kg	ED_{50}, mg/kg	AAI LD_{50}/ED_{50}
Napelline	88.0	10.0	8.8
12-Epinapelline (**2**)	82.0	8.0	10.3
12-Acetyl-12-epinapelline (**1**)	40.5	6.1	6.6
Quinidine	66.9	15.4	4.3
Novocainamide	138.0	40.7	3.4

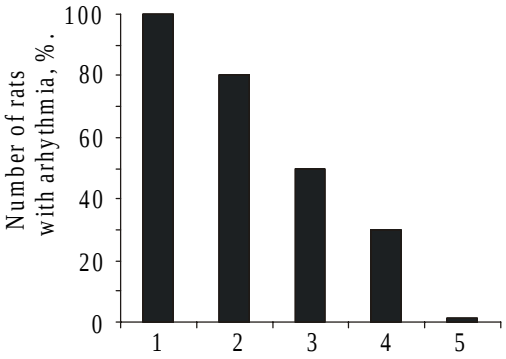

Fig. 2. Effect of 12-acetyl-12-epinapelline on rat arhythmia from aconitine. Control, H_2O + aconitine, 12 μg/kg (1); administration of 12-acetyl-12-epinapelline (mg/kg): 2.5 (2), 5.0 (3), 10.0 (4), 15.0 (5).

EXPERIMENTAL

Deactivated Al_2O_3 was used for column chromatography. The purity of the compounds was checked using thin layers of chromatographic-grade Al_2O_3 and benzene:ethanol (20:1) and ether:hexane (3:1). IR spectra were recorded on a Fourier-IR spectrometer (Perkin—Elmer, model 2000) in KBr disks; PMR spectra, on a BS-567A instrument (Tesla) at 100 MHz in $CDCl_3$ with HMDS as the solvent and internal standard; mass spectra, in a MS 25 RF (Kratos, Great Britain).

Separation of Crude Mother Liquor of Aconitine. Crude mother liquor of aconitine (15.7 g) was dissolved with boiling in acetone and left at room temperature. The resulting crystalline precipitate was separated and recrystallized from acetone to afford karacoline (2.75 g), mp 178-180°C.

The mother liquor from karacoline separation was placed on a column of deactivated Al_2O_3 (1:30). Eluents were benzene (fractions 1-12) and benzene:ethanol (100:1) (13-18), (50:1) (19-22), (25:1) (23-27), and (10:1) (28-36). Fraction 17 afforded **2** (0.64 g). Fractions 19-24 gave songorine (1.49 g); fractions 26-30, napelline (3.0 g).

Pharmacologic Tests. The biological activity of **1** was investigated in tests on white mongrel mice of mass 20-22 g and white rats of mass 230-280 g. The nature of resorption and acute toxicity was determined in unanesthetized mice. The preparation was administered through a tail vein. The effect of i.v. administration of the preparation on cardiac bioelectric activity was studied in experiments in rats. EKG scans were recorded in the II standard lead at a recorder rate of 50 mm/s.

Antiarhythmic activity of the preparation was investigated using the cardiac arhythmia model elicited by aconitine (10-15 μg/kg) in rats anesthetized with sodium pentobarbital (40 mg/kg, i.p.). Aconitine hydrochloride (Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan) was administered i.v. at doses of 12-15 μg/kg. In control experiments, i.v. administration of aconitine at these doses was performed in 100% of the cases to develop allorhythmic extrasystolic arhythmia of greater than 60-min duration. The antiarhythmic action of the preparation was judged from its ability

to protect (prophylactic action) or diminish the arhythmic action of aconitine and restore normal sinusoidal rhythm. The preparation was tested at doses of 2.5, 5.0, 10.0, 15.0, and 20.0 mg/kg. Each dose was tested in 5-6 animals.

The toxicity and antiarhythmic activity of **1** was compared with the action of napelline and **2** and with the effectiveness of the standard class-I antiarhythmics quinidine and novocainamide. The average lethal dose (LD_{50}) was determined by the Litchfeld—Wilcoxon method [10]; the average effective antiarhythmic dose, graphically. The range of antiarhythmic activity was judged from the ratio of LD_{50} to ED_{50}, which was designated the antiarhythmic index (AAI).

X-ray Structure Analysis. Crystals of **1** ($C_{24}H_{35}NO_4$) were grown as transparent elongated prisms. A crystal of approximate dimensions 0.35 × 0.55 × 0.85 mm was selected for the XSA. Cell constants of **1** were found and refined on a Stoe Stadi-4 diffractometer at 295 K. Crystals of **1** are orthorhombic, a = 11.889(7), b = 13.213(7), c = 13.326(5) Å, V = 2093(2) Å^3, d_{calc} = 1.274 g/cm^3, absorption coefficient μ = 0.085 mm^{-1}, space group $P2_12_12_1$, Z = 4. Intensities of reflections were measured on the same diffractometer by $\omega/2\theta$-scanning using Mo Kα-radiation (graphite monochromator), $2\theta \le 55°$. Intensities were initially processed using the program XRED [11]. Absorption corrections were not applied.

The structure was solved by direct methods using the automated program SHELX-86 [12] and refined by successive isotropic-anisotropic least-squares methods using the program SHELXL-93 [13] for nonhydrogen atoms. Coordinates of H atoms of the hydroxyls were calculated from a difference electron-density synthesis; of the remaining H atoms, from geometric considerations. All H atoms were refined isotropically. The final agreement factors are $R_1(F)$ = 0.0640 (wR_2 = 0.1427) for 2044 reflections with $I > 2\sigma(I)$ and 0.0945 (wR_2 = 0.1684) for all 2726 independent reflections used in the final refinement cycle. Coordinates of nonhydrogen atoms and equivalent anisotropic thermal parameters from the final least-squares refinement cycle are deposited in the Cambridge Structural Database [14].

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