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CONFORMATIONAL ANALYSIS OF LAPPACONITINE AND HETERATISINE

B. Tashkhodzhaev

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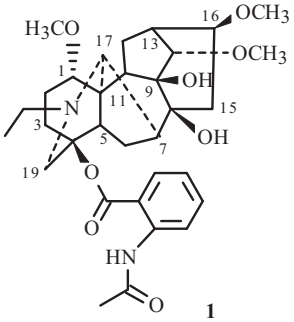
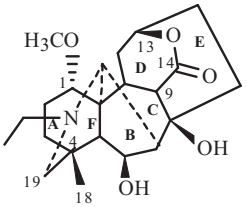
The conformation of the carbon skeleton in diterpenoid alkaloids of the lappaconitine, heteratisine, and lycoctonine types was analyzed as a function of the nature of the substituents and intramolecular H-bonds and their states in the crystal based on x-ray structure analyses of lappaconitine and heteratisine and data in the Cambridge Crystallographic Data Centre. Ring A in these diterpenoid alkaloids adopted the boat or chair conformation depending on the presence or absence of an intramolecular H-bond between atoms of the skeleton N and O in the C1 position, respectively. The other rings C, D, and F of the framework did not undergo substantial conformational changes whereas rings B and E showed slight distortion that converted them to other similar canonical forms.

Keywords: diterpenoid alkaloids, lapaconitine, heteratisine, x-ray structure analysis.

Studies of diterpenoid alkaloids and their analogs found that many C_{18}-bis-nor-diterpenoid alkaloids of the lappaconitine (**1**) and heteratisine (**2**) types have characteristically pronounced anti-arrhythmic activity [1, 2].



1



2

The carbon skeleton of the bis-nor-diterpenoid alkaloid lappaconitine differs from that of lycoctonine by the lack of a C18 atom; from heteratisine, by the presence of a cyclopentane ring instead of a lactone ring. They have three-dimensional frameworks with fluxional hydroxy substituents.

According to data in the Cambridge Crystallographic Data Centre (CCDC), the molecular structures of about 60 diterpenoid alkaloids with the lycoctonine, lappaconitine, and heteratisine carbon skeletons have until now been solved. An analysis of the literature 3D data (based on the CCDC using the program Mercury [3]) indicated that the lycoctonine (like lappaconitine) carbon skeleton, which consists of six prinicpal rings, is rather rigid and is not distorted substantially with respect to the positions, substituent orientations (hydroxyls), and nature of intramolecular H-bonds with the exception of ring A (atoms C1-5 and C11).

The conformation of ring A in these alkaloids depends on the nature of the C1 substituent. If C1 has an α-OH group, then an intramolecular O–H...N H-bond is formed between the hydroxyl H atom and the N atom (a six-membered pseudo-ring is formed). Ring A adopts the boat conformation. If the C1 α-OH is substituted by CH_3-, CH_3CO-, C_6H_5CO-, and other groups, or the C1 OH group has the β-orientation, then this intramolecular H-bond is missing and ring A has the chair conformation. Crystals of the alkaloids salts (water-soluble forms used in medical practice) exhibit only the boat conformation for ring A. In these instances, the framework N atom is protonated and ring A always adopts the boat conformation due to the formation of an intramolecular N^+–H...O–C1 H-bond.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax: (99871) 120 64 75, e-mail: tashkhodjaev@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 356–359, May–June, 2010. Original article submitted October 20, 2009.

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TABLE 1. Geometry of H-bonds (Å and deg) in Crystals of **1** and **2**

D–H...A	D–H	H...A	D...A	D–H...A
		Molecule **1**		
O6-H...O5	0.82	2.25	2.671	113
O5-H...O7	0.82	2.26	2.858	130
N2-H…O3	0.86	1.98	2.678	138
Ow-H…O5	–	–	2.785	–
		Molecule **2**		
O3-H…O2	0.82	2.04	2.691	136
O2-H...O3 (*–x; –1/2+y; –z*)	0.82	2.18	2.998	174

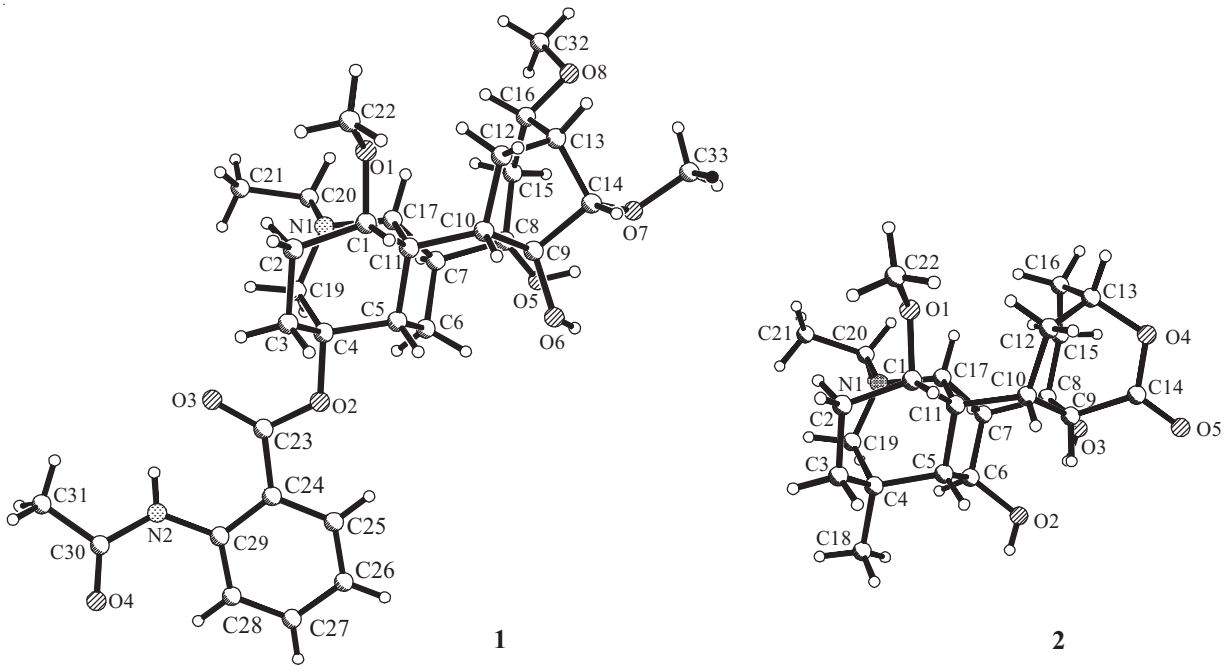

Fig. 1. Molecular structure and atomic numbering in **1** and **2**.

However, the CCDC database has a single example where the aforementioned trends are not observed. The C14 hydroxyl H atom (external transformed molecule) in the crystal structure of 15-*epi*-isodelphonine [4] forms simultaneously a bifurcated intermolecular H-bond with the N and O1 atoms of the original molecule (N...H...O1 H-bond is formed). In other words, this external H atom is an intermediate in the formation of an intramolecular six-memered pseudo-ring, as a result of which ring A adopts the boat conformation.

An analogous adoption of the boat or chair conformation of ring A is observed also in heteratisine alkaloids. However, only two of the crystallographically known five heteratisines in the CCDC database [5–7] have 3D data. The alkaloids 8-acetylheterophyllisine [6] and heteratisine hydrobromide monohydrate [7] contain a C1 methoxyl in the carbon skeleton. Ring A of the base (8-acetylheterophyllisine) adopts the chair conformation whereas the salt (heteratisine) forms an intramolecular N^+–H...O H-bond, because of which ring A adopts the boat conformation. This was noted in diterpenoid alkaloids of the lappaconitine (lycoctonine) group. Other rings of the carbon skeleton in both molecules are geometrically identical despite the fact that the heteratisine salt contains an intramolecular H-bond in addition to an intermolecular H-bond, in contrast with 8-acetylheterophyllisine.

The trends noted above for the conformation of ring A were mentioned earlier by us [8] with respect to the lycoctonine skeletion based on several examples. It seemed interesting for further expansion of the experimental data set to examine for conformation of ring A and other rings of the framework in alkaloid bases lappaconitine and heteratisine, the structures of which were established earlier as the hydrobromide monohydrates [9, 7]. Therefore, we performed an x-ray structure analysis (XSA) of crystals of these compounds isolated from *Aconitum talassicum* M. Pop. and *A. zeravschanicum* Steinb., respectively.

Figure 1 shows the x-ray molecular structure of lappaconitine (**1**) and heteratisine (**2**). The molecule of **1** has O–H...O intramolecular H-bonds for C8 and C9 hydroxyls and for the C9 hydroxyl and the C14 methoxyl. Although these intramolecular H-bonds were missing in the salt of **1** [9], the active H atoms were involved in the formation of intermolecular H-bonds. This difference could lead to conformational changes of the rings in the carbon skeleton (see below). The intramolecular N–H...O H-bond between the amine (N2–H) and carbonyl (C23=O3) in the acetylanthranoyloxyl fragment (on C5) was retained in both crystal forms (base and salt). Table 1 gives the parameters for these H-bonds.

Heteratisine (**2**) also had an intramolecular O–H...O H-bond between the C6 and C8 hydroxyls (Table 1) that were missing in its 6-benzoyl derivative [10] but were retained in heteratisine salt [7]. The effect of these H-bonds on the conformation of the heteratisine skeletion is also interesting (see below).

Crystals of lappaconitine were the hemihydrate. The water of crystallization was located in a special position with population 0.14, i.e., the water was present at an alkaloid:water ratio of about 7:1. These water molecules were bonded to O atoms of the corresponding hydroxyl (O5–H) through an intermolecular H-bond (Table 1).

There was also an intermolecular O–H...O H-bond in the crystal of **2** between the C6 and C8 hydroxyls transformed by 2_1 screw axes along the *b* axis (Table 1).

Ring A in the carbon skeleton of **1** adopted an ideal chair conformation (deviation of atoms from the plane of four atoms less than ±0.008 Å); five-membered ring B (atoms C5-7, 11, 17), a 17α,11β-half-chair. Six-membered ring C (atoms C7-11, 17) was found as a twisted chair with a two-fold symmetry axis preserved. A visual comparison of the conformations of rings B found in other related alkaloids showed that ring B was distorted from a 17α-envelope to an 11β-envelope or adopted an intermediate conformation as a 17α,11β-half-chair (easily inverted conformations). Ring C in the known alkaloids retained the canonical chair conformation although the degree of twisting of ring C from the ideal chair was slightly different in these alkaloids.

Five-membered ring D in **1** was close to a 14β-envelope (±0.018 Å) with C14 deviating from the plane of the other atoms by 0.700 Å. This conformation of ring D was found in all literature examples. Ring E (atoms C8, 9, 13-16) in **1** adopted the chair conformation (±0.067 Å) with C14 deviating (0.923 Å) from the plane of the other five. However, there are many examples in the literature where this ring adopted a flattened boat around C15 and sometimes a flattened chair around C15 [11]. The nature of the substituents on the ring itself or its closest neighbors was probably responsible for this slight change of ring E from the chair conformation. Heterocycle F (atoms C4, 5, 11, 17, 19, N) in **1** had the chair conformation (±0.040 Å) with flattening toward C19. Ring F in all related alkaloids adopted either the chair or twisted chair conformation except for those instances where the ring atoms were involved in formation of new oxazoline rings.

In heteratisine (**2**), the carbon framework differed from that of lycoctonine (as noted above) by the presence of six-membered δ-lactone ring D (atoms C9, 10, 12, 13, O4, C14) instead of the cyclopentane. Therefore, ring E became seven-membered. Ring A in **2** adopted the chair conformation like that found in 6-benzoyl heeteratisine [10]. However, this ring in heteratisine salt had the boat conformation. The conformation of rings B-F in **2** did not differ substantially from those observed in its salt [7] and in derivatives [10, 11]. Ring B in these compounds adopted a stable 17α-envelope conformation; six-membered rings C, chair; D, boat; and seven-membered ring E and heterocycle F, a slightly distorted chair.

In summarizing our data and that in the literature regarding the conformations in diterpenoid alkaloids with the lappaconitine, heteratisine, and lycoctonine skeletons, we note that ring A in them can adopt the boat or chair conformation depending on the presence or absence of an intramolecular H-bond between atoms of the skeleton N and C1 O, respectively. Five-membered ring B is found in three easily converted conformations, 17α-envelope, 11β-envelope, and 17α,11β-half-chair. Six-membered ring C had the chair conformation regardless of the position and presence of substituents although it was slightly distorted from the ideal conformation. Heterocycle F including the N atom was found in the chair conformation with slight distortions from the ideal conformation (except for instances where the ring F atoms were involved in formation of new rings). Ring D in lycoctonine (lappaconitine) alkaloids adopted a stable 14α-envelope conformation. Ring E in them had mainly the 14α-chair conformation although C15 deviated slightly from the plane of the other four (deviation from the chair conformation) depending on the effect of the closest substituents. This could lead to a slightly flattened chair or boat because of steric effects. Rings D and E in heteratisine alkaloids adopted the boat and chair conformations, respectively. Thus, rings C, D, and F of the framework did not undergo substantial conformational changes whereas rings B and E had slight distortions with transitions into other similar canonical conformations.

TABLE 2. Principal Crystallographic Parameters and Characteristics of X-ray Structure Analysis

Structure	**1**	**2**
Molecular formula	$C_{32}H_{44}N_2O_8 \cdot 0.14H_2O$	$C_{22}H_{33}NO_5$
MW, g/mol	584.69	391.49
Space group	$P3_121$	$P2_1$
Z	6	2
a, Å	10.8074(6)	9.106 (2)
b, Å	10.8074(6)	10.610 (2)
c, Å	44.179(2)	10.085 (2)
α, °	90	90
β, °	90	92.5 (1)
γ, °	120	90
V, Å^3	4469 (1)	973.5 (3)
ρ, g/cm^3	1.304	1.336
Cryst. size, mm	0.45 × 0.30 × 0.25	0.80 × 0.50 × 0.35
Scan region, 2θ°	4.00 ≤ θ ≤ 75.58	2.02 ≤ θ ≤ 25.01
μ_{exp}, см$^{-1}$	0.763	0.094
Number of reflections	4272	1813
Number of refl. with $I > 2\sigma$ (I)	3079	1700
R_1 ($I > 2\sigma$ (I) and total)	0.0925 (0.1182)	0.0359 (0.0405)
wR_2	0.2220 (0.2591)	0.0959 (0.1023)
COOF	1.017	0.973
Difference ED peaks, *e* Å^{-3}	0.38 and –0.34	0.17 and –0.14

EXPERIMENTAL

Crystals of **1** were grown from EtOH (mp 217–218°C). Unit-cell constants were determined and refined on an Xcalibur diffractometer (Oxford Diffraction) (T = 293 K, graphite monochromator) [12]. Table 2 gives the principal crystallographic parameters and calculations for the crystal of **1**. A three-dimensional data set of reflections for **1** was collected on the same diffractometer using Cu K_α-radiation. Absorption corrections were applied using the Multi-scan method [12].

Crystals of **2** were also grown from EtOH (mp 251–256°C, dec.). Unit-cell constants for **2** were determined and refined on a Stoe Stadi-4 diffractometer (T = 293 K, graphite monochromator) [13]. A three-dimensional data set of reflections was collected on the same diffractometer using the ω/2θ-scanning method and Mo K_α-radiation. Absorption corrections were not applied. Table 2 gives the principal crystallographic parameters and calculations for the crystal of **2**.

The structures were solved using direct methods and the SHELXTL Plus 5.0 program set [14]. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (on F^2). Positions of H atoms were found geometricallly and refined with fixed isotropic thermal parameters $U_{iso} = nU_{eq}$, where n = 1.5 for methyls and 1.2 for others and U_{eq} is the equivalent isotropic thermal parameter of the corresponding C, N, or O atom. H atoms of hydroxyls were found experimentally from a difference electron-density (ED) synthesis and refined isotropically and bound to the corresponding O atom.

An isolated peak in a special position (on a 3_1 screw axis along the crystallographic *b* axis) was observed in the final LS refinement stages of **1**. The peak was positioned based on refinement of the multiplicity (population of the position) and intermolecular contacts (H-bonds) as the O atom of a water of crystallization with multiplicity 0.14.

Data from the XSA were deposited as CIF files in the Cambridge Crystallographic Data Centre (CCDC 751807 and 751808 for **1** and **2**, respectively).

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