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STRUCTURE AND BIOLOGICAL ACTIVITY OF DEMETHYLENELDELIDINE AND ITS DIHYDRO-γ-LACTONE

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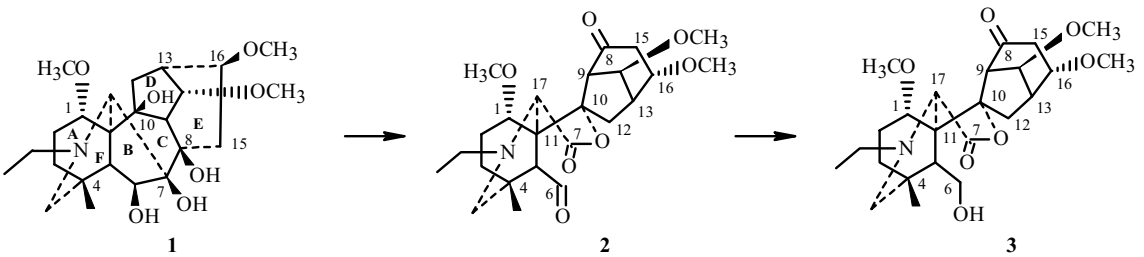
*An excess of periodic acid was reacted with the diterpenoid alkaloid demethyleneldelidine (**1**) to produce a γ-lactone (**2**) in which the carbonyl at the C6-position was selectively reduced by an excess of $NaBH_4$. The conformation of the rings, configuration of asymmetric and spiro centers, and the nature of intramolecular H-bonds in the diterpenoid alkaloid demethyleneldelidine and its reduction product **3** were analyzed by an x-ray structure analysis. The spiro center at C10 in **3** had the* S*-configuration. The other asymmetric centers retained the absolute configuration characteristic of alkaloids with the lycoctonine carbon skeleton. The pharmacological properties did not change significantly despite the large difference in the carbon skeletons of γ-lactone **3** and starting **1**.*

Key words: diterpenoid alkaloid, demethyleneldelidine, γ-lactone, XSA, pharmacological properties.

Studies of structure–activity relationships of lycoctonine and heteratisine alkaloids and their analogs showed that the antiarrhythmic activity (AA) of these compounds depended on the nature of the C6 substituent [1, 2]. Thus, the AA of eldeline, a lycoctonine-type 6-acetoxy compound, is more than two times greater than that of eldelidine, which is significantly inferior to 6-benzoyleldelidine in this respect [1]. An analogous situation was observed upon comparing effective doses of 6-hydroxy-, 6-acetoxy-, and 6-benzoyloxyheteratisine compounds [2]. The last compound and 6-*O*-benzoyleldelidine had greater AA and broader therapeutic action than known class I antiarrhythmics of the quinidine group (quinidine, novocainamide, ritmilen, etmozin, lidocaine) that are used in medical practice. The high AA of 6-*O*-benzoylheteratisine may be due to the δ-lactone ring in its molecule. Therefore, it seemed interesting to transform available lycoctonine-type alkaloids into aminoalcohols with a lactone ring because compounds with higher pharmacological activity, in particular AA activity, might be found among their ester derivatives.

Demethyleneldelidine (**1**), which was first prepared synthetically from eldeline and later observed in *Delphinium dictyocarpum* DC. [3–6], was transformed to γ-lactone product **2** with an aldehyde and carbonyl [7, 8]. Then, **2** was reduced by $NaBH_4$ to the corresponding aminoalcohols with a γ-lactone ring because this ring should not be affected under these conditions. Reaction of **2** with $NaBH_4$ formed a complex mixture of aminoalcohols because of the presence in the starting compound of two reactive centers.

Because of this, we oxidized demethyleneldelidine with an excess of periodic acid and produced chromatographically pure semi-crystalline **2**, reaction of which with an excess of $NaBH_4$ gave a mixture of products in which the dominant one was **3**, mp 215–217°C (dec., MeOH). The yield of **2** was 69.2%; of its reduction product **3**, 62.0% of theoretical.



1 **2** **3**

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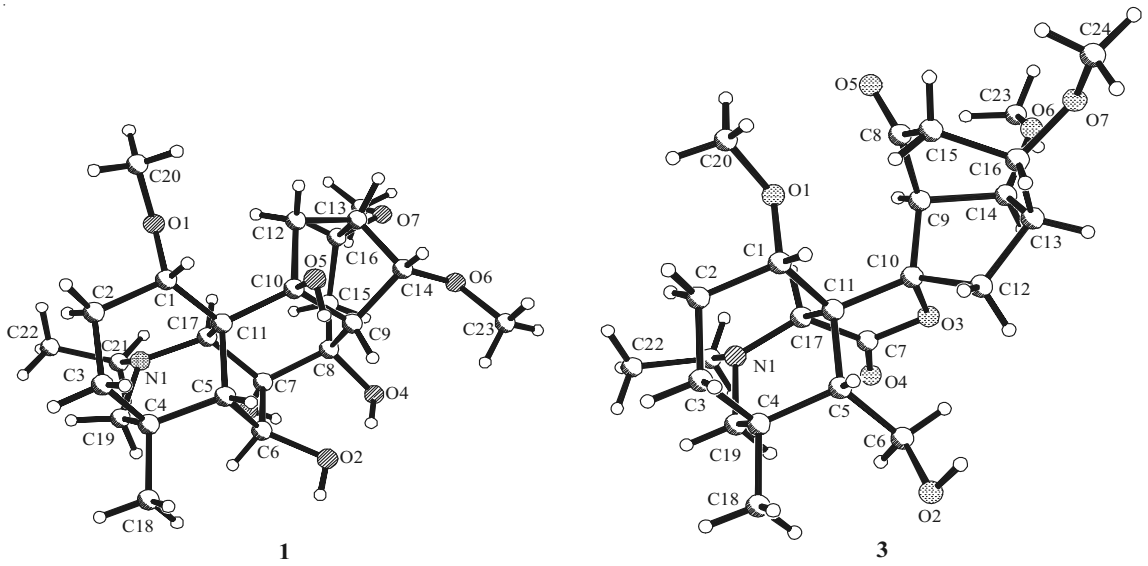

Fig. 1. Molecular structure and atomic numbering of **1** and **3**.

The carbon skeleton in the γ-lactone underwent substantial changes compared with lycoctonine. The three asymmetric centers C6, C7, and C8 disappeared from starting **1** and the new spiro center C10 appeared. It should be noted that a structure for the γ-lactone of demethyleneldelidine was proposed in which the relative configuration of these substituents was not resolved. The interest in the molecular structure of **3** is obvious considering the conformational and configurational issues and the question of the absolute configuration of the asymmetric (spiro-) centers in the new γ-lactone backbone. These problems were solved reliably by an x-ray structure analysis (XSA) of **3**. The structure of starting demethyleneldelidine (**1**), which was isolated from *D. dictyocarpum* (mp 98–100°C), was elucidated because it was proposed earlier on the basis of physicochemical and spectral data [3–6].

According to the XSA, **3** was the dihydroproduct of γ-lactone **2** in which the C8 carbonyl was unaffected. A spiro center of absolute configuration *S* was formed on going from demethyleneldelidine to the γ-lactone. In order to portray correctly the structural formula of the γ-lactone in **2** and **3**, the bicyclo[1.2.3]octane (D/E ring) system should be rotated around the 3-azabicyclo[1.3.3]nonane (rotation around the C10–C11 bond). Considering this, the portrayed orientation of the C14 and C16 methoxyls in the newly formed pentacyclic system of **3** should be changed to the opposite one, because of which their relative configurations will be designated as α and β from hereon.

The XSA showed that crystals of **1** were the dihydrate, i.e., the crystal contained two waters per base molecule. This indicates that the natural demethyleneldelidine studied by us was identical to the synthetic material obtained previously also as the dihydrate (mp 97–99°C) [3]. This means that demethyleneldelidine tends to include water molecules upon forming crystals.

Crystals of the demethyleneldelidine transformation products that were obtained for XSA from MeOH (**3a**) and EtOH (**3b**) were different (mp 215–217°C and 202–205°C, respectively). The unit-cell constants were also slightly different (Table 1). One of the crystallographic axes (*c*) in **3a** was noticeably longer (by 1.59 Å) than that in **3b** (so that the volume was greater by 114 $Å^3$). Therefore, XSA were performed for both single crystals. The XSA showed that the crystal of **3a** contained a MeOH of crystallization; **3b**, no solvent molecule (EtOH). This was the reason for the smaller volume of the unit cell and the difference in the melting point although the molecular structure (carbon skeleton with substituents) of γ-lactone-containing **3** was practically identical in both crystal forms. Fluxional groups in the C1, C5, and C14 positions also had the same orientations. A difference was observed only in the orientation of the C16 methyl (O–CH_3).

Figure 1 shows the structures of **1** and **3** from XSA with approximately the same projection of ring A (the molecule of **3** shown in Fig. 1 corresponds to the structure found in **3a**).

Figure 1 shows that the conformation of rings and their fusion (A/B-*cis*, C/D-*cis*) in **1** are traditional for diterpenoid alkaloids with the lycoctonine carbon skeleton. Rings A (C1-5,11) and C (C7-11,17) had the chair conformation; D (C9,10,12–14), the 14β-envelope; E (C8,9,13–16), the 14β-half-chair; and F (C4,5,11,17,19,N1), a slightly distorted chair. The positions of the hydroxyls were as follows. The C6, C7, C8, and C10 hydroxyls and the C16 methoxyl had the β-orientation; the C1 and C14 methoxyls, the α-orientation. The position of the hydroxyls favored formation of intramolecular H-bonds by the following sequential scheme of acceptors and donors: O3–H...O4–H...O2–H. The parameters of these H-bonds were O3...O4 and O4...O2, 2.760 and 2.666 Å; H...O4 and H...O2, 2.35 and 1.91 Å; O3–H...O4 and O4–H...O2, 120 and 148°, respectively.

TABLE 1. Toxicity and Comparative Antiarrhythmic Activity of Eldeline, Eldelidine, Demethyleneldelidine, and Their Derivatives

Compound	LD_{50}	ED_{50}	AAI LD_{50}/ED_{50}
	i/w, mg/kg		
6-*O*-Benzoyleldelidine	16.1	0.67	24.0
Eldeline (6-*O*-acetyleldelidine)	136.0	10.2	12.7
Eldelidine	235.0	25.4	9.3
Demethyleneldelidine	230.0	30.0	7.7
Dihydro-γ-lactone of demethyleneldelidine	240.0	25.1	9.6
Quinidine	66.9	15.4	4.3
Novocainamide	138.0	40.7	3.4

The molecule of **3**, despite the great change of the carbon skeleton compared with **1**, retained (in crystal forms **3a** and **3b**) the absolute configuration of all other similarly numbered asymmetric centers. The chair conformation was retained in ring A. The lactone ring (C7,10,11,17,O3), which formed instead of rings B and C, was *cis*-fused with ring F and had the 11α-envelope conformation. This ring was fused to rings D and E through spiro center C10. Ring D had the 14α-envelope conformation. Ring E was an unsymmetrical chair flattened toward C15. Ring F had the chair conformation.

All intermolecular H-bonds in the crystalline dihydrate **1** involved waters of crystallization. The H atoms of one water molecule were bonded to unshared pairs of C14 and C16 methoxyl O atoms. The parameters for Ow2–H...O6 and Ow2–H...O7 H-bonds [O...O and H...O distances (Å) and O...H–O angle (deg)] were 2.954, 2.40, 132 and 2.986, 2.32, 150. However, the other water molecule was involved in four intermolecular H-bonds, in two as an acceptor and in the other two as a donor. The following H-bonds were formed: Ow1–H...Ow2 (2.756, 1.94, 174); Ow1–H...O4 (2.796, 1.94, 157); Ow1...H–O2 (2.770, 2.01, 177); Ow1...H–O5 (2.900, 2.11, 167). The arrangement of these corresponded to a distorted tetrahedron. Molecules transformed by translational symmetry elements and a 2_1 screw axis were networked in the *ab*0 plane.

The molecular packing was isostructural in **3a** and **3b**. The MeOH of solvation was involved in **3a**. Its hydroxyl H atom formed an intermolecular H-bond with the O6–CH_3 and O7–CH_3O atoms. The parameters of these bonds (3.002, 2.90, 94 and 2.911, 2.82, 93, respectively) indicated weak interactions. However, the other intermolecular H-bond of the C=O...H–O type (3.029, 2.09, 161) between the C8 carbonyl of the starting molecule and the C6 hydroxyl that was transformed by a 2_1 screw axis formed an infinite chain along the *a* axis. An analogous H-bond was retained in the molecular packing of **3b**. However, the parameters of this H-bond had the values 2.972, 2.12, 166.

Pharmacological Studies. Comparative studies of **1** and the dihydro-γ-lactone prepared from it, demethyleneldelidine (**3**), were performed in order to find the effect of changing the carbon skeleton on the biological activity.

The resorptive activity of **3** did not differ substantially from that of **1** upon intravenous injection to white mice. Both compounds at subtoxic and lethal doses caused adynamia, weakening of skeletal muscle, respiratory depression, exophthalmus, asphyxial cramps, and respiratory arrest. Compound **3** was less toxic than **1** according to acute toxicity parameters. However, the difference was not statistically significant ($p > 0.05$) (Table 1).

In acute tests in anesthetized cats, **1** and **3** at doses of 20–40 mg/kg (i/w) caused brief lowering of arterial pressure by 15–30 mm Hg for 3–10 min. The EKGs showed cardiac contractions at reduced frequency and lengthening of RR, PQ, and QT invervals that were characteristic of class I quinidine-like antiarrhythmics.

The hypotensive activity of **1** and **3** were due to a short-term ganglio-blocking action. The toned third eyelid of a cat was weakened by 20–40% at these doses of **1** and **3** due to continuous electrical stimulation of the pre-ganglic terminus of the upper cervical sympathetic nerve. Compounds **1** and **3** did not affect or slightly intensified the pressor activity of adrenaline and decreased effects caused by cytisine.

Compounds **1** and **3** at concentrations up to 10^{-4} g/mL were equally weakly active and had no significant effect on spasm caused by acetylcholine, histamine, and barium chloride in *in vitro* tests on isolated rat small intestine and frog abdomen muscle.

Compounds **1** and **3** at doses of 15–40 mg/kg had a protective and short-term antiarrhythmic action in a cardiac arrhythmia model caused by aconitine in rats. Compound **3** was slightly better than **1** ($p > 0.05$) according to antiarrhythmic activity and therapeutic index (LD_{50}/ED_{50}). Both compounds had antiarrhythmic activity inferior to that of quinidine but better therapeutic indices. The antiarrhythmic activity and therapeutic effects were better than those of novocainamide.

TABLE 2. Principal Crystallographic Parameters and Characteristics of X-ray Structure Analysis of **1** and Its Transformation Product in Two Crystal Forms (**3a** and **3b**)

Structure	**1**	**3a**	**3b**
Molecular formula	$C_{24}H_{39}NO_7 \cdot 2H_2O$	$C_{24}H_{37}NO_7 \cdot CH_3OH$	$C_{24}H_{37}NO_7$
MW/g·mol^{-1}	489.59	483.59	451.55
Space group	$P2_1$	$P\ 2_12_12_1$	$P2_12_12_1$
Z	2	4	4
a, Å	10.387 (2)	7.832 (2)	8.024 (8)
b, Å	8.127 (2)	13.212 (3)	13.181 (11)
c, Å	15.349 (3)	23.703 (5)	22.112 (18)
β	107.76 (3)	90	90
V, Å^3	1233.9 (4)	2453 (1)	2339 (4)
ρ, g/cm^3	1.318	1.310	1.282
Crystal size (mm)	0.80 × 0.60 × 0.40	0.80×0.65×0.55	0.75 × 0.50 × 0.30
Scan range 2θ	2.06 ≤ θ ≤ 27.49°	1.72 ≤ θ ≤ 27.48°	1.80 ≤ θ ≤ 25.00°
μ_{exp} (cm^{-1})	0.100	0.097	0.093
Number of reflections	3041	3196	2364
Number of refl. with $I>2\sigma$ (I)	2771	2715	1653
R_1 (I>2σ (I) and total)	0.0392 (0.0455)	0.0549 (0.0691)	0.0663 (0.1143)
wR_2	0.0920 (0.0971)	0.1298 (0.1414)	0.1215 (0.1466)
GOOF	1.069	1.090	1.185
Electron-density difference peaks, *e* Å^{-3}	0.24 and –0.18	0.30 and –0.32	0.21 and –0.21

Thus, a comparison of the biological activities of **1** and **3** showed that there were no substantial changes in the resorptive activity, degree of toxicity, EKG, and antiarrhythmic activity despite the significant change in the carbon skeleton of **3**.

We found earlier that the toxicity, antiarrhythmic activity, and cardio-selectivity in a series of very close analogs of **1** increased in the order eldelidine < eldeline (6-*O*-acetyleldelidine) < 6-*O*-benzoyleldelidine. Data on the toxicity and antiarrhythmic effectiveness of these alkaloids are given in Table 1 for comparison. The pharmacological properties and toxicity of **3** were similar to those of **1** and eldelidine. The questions of whether a similar dependence between the structure and antiarrhythmic activity in a series of C-6 *O*-acyl-substituted of **3** will be observed and how pronounced it is will be the subject of the next report (remain open and require further research).

EXPERIMENTAL

The purity of compounds was checked by TLC over Al_2O_3 using $CHCl_3$ and $CHCl_3$:MeOH (20:1). Melting points were determined on a Boetius stage.

Preparation of Demethyleneldelidine γ-Lactone (2). Compound **1** (1.0 g) was treated with a solution of $HIO_4 \cdot 2H_2O$ (1.6 g, 3 M) in water (50 mL). The resulting solution was left for 2 d at room temperature, made basic with Na_2CO_3, and shaken with $CHCl_3$. The solid obtained after drying over Na_2SO_4 and distilling solvent was worked up with MeOH to give chromatographically pure **2** (0.63 g).

Preparation of Dihydroproduct of Demethyleneldelidine γ-Lactone (3). A solution of **2** (0.5 g) in a mixture of MeOH (30 mL) and H_2O (3 mL) was stirred and treated with $NaBH_4$ (0.5 g) over 1 h. The excess of solvent was removed. The solid was diluted in HCl solution (50 mL, 10%). The acidic solution was washed with Et_2O, made basic with Na_2CO_3, and shaken with $CHCl_3$. The solid obtained after drying over Na_2SO_4 and distilling solvent was worked up with MeOH to give **3** (0.31 g), mp 215–217°C.

X-ray Structure Analysis. Unit cell constants of **1**, **3a**, and **3b** were determined and refined on a Stoe Stadi-4 diffractometer (T = 293 K, graphite monochromator). A three-dimensional dataset of reflections was obtained on the same diffractometer by ω/2θ-scanning using Mo K_α-radiation. Absorption corrections were not applied. Table 2 lists the principal parameters of the x-ray structure analysis and calculations.

The structures were solved by direct methods using the SHELXTL Plus 5.0 program set [9]. All nonhydrogen atoms were refined by anisotropic full-matrix least-squares methods (over F^2). Positions of H atoms were found geometrically and refined using 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 and waters of crystallization were found experimentally from a difference electron-density synthesis and refined isotropically by linking to the corresponding O atom.

Data from the x-ray structure analyses were deposited as CIF files in the Cambridge Crystallographic Data Centre (CCDC 728622, 728623, 728624 for **1**, **3a**, and **3b**, respectively).

Pharmacological tests were performed on white mongrel mice (18–22 g), rats (220–250 g), and cats (2–3 kg). Details of the tests have been published [10, 11].

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