STRUCTURE OF THE NEW UNUSUAL DITERPENE ALKALOID ACOFINE FROM *Aconitum karakolicum*

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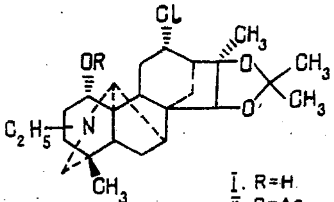
The structure of the new diterpene alkaloid acofine, isolated from tubers of Aconitum karakolicum, has been shown by the x-ray structural method (diffractometer, CuK_{α}, 1250 reflections, direct method, R = 0.071). The IR, mass, and PMR spectrum of acofine and its monoacetate are considered. Acofine is the first chlorine-containing diterpene alkaloid, and also the first alkaloid of the napelline type with a 15,16β-acetonide group.

The present paper describes the structure of the new alkaloid acofine, which was first isolated from tubers of Aconitum karakolicum Rapaics twenty years ago and was called "the base with mp 159-160°C" [1].

Acofine (I) has the composition $C_{25}H_{38}NO_3Cl$ (HRMS M^+ 435.25502). The IR spectrum of the hydrochloride of the base showed the absorption band of a hydroxy group at 3250 cm^{-1}. Its PMR spectrum contained the signals of four tertiary C-methyl groups and of an N-ethyl group. In the mass spectrum there were isotopic peaks showing the presence of a chlorine atom in the molecule. The structure of the acofine molecule has been established by the x-ray structural method.



I. R=H
II. R=Ac

The spatial structure of the molecule of (I) in a projection on the plane of the C1C4C9 atoms is shown in Fig. 1. It can be seen that acofine contains a napelline skeleton with 15,16β-acetonide and 16α-methyl groups. The molecule has an α-oriented hydroxy group in the C1 position and a similarly orientated chlorine atom at C12.

Ring A (the C1—C5 and C10 atoms) assumes a chair conformation with an accuracy of ±0.06 Å, with deviations of the C2 and C5 atoms (—0.20 Å and 0.67 Å, respectively), but in another aspect it has a twist-chair conformation with C_2 symmetry, as is shown by the asymmetry parameters ΔC_2 (1-2, 4-5) = 4.1° and ΔC_S (2,5) = 14.0°. The distortion of ring A is perhaps partially explained by the strong thermal vibrations and the consequent inaccuracy in the position of the C2 atom (Table 2). The boat conformation is adopted by the six-membered rings B (the C5-C10 atoms), with appreciable distortion (±0.095 Å), and C (the C8, C9, and C11-C14 atoms), in practically ideal from (±0.002 Å). The five-membered ring D (the C8, C13-C16 atoms) has the conformation of a C14α-envelope (±0.1 Å), and ring E (the C15, C16, O2, O3, and C23 atoms) is a flattened C23α-envelope (±0.02 Å). However, rings H (the C5-C7, C20, and C10 atoms) and K (the C7-C10 and C20 atoms) assume half-chair conformations with deviations of the C10 and C20 atoms (in ring H) and the C7 and C20 atoms (in ring K) in different directions. The piperidine ring F (the C4, C5, C20, C18, and N atoms) has a chair conformation. The linkage of rings A/B is trans, B/C cis, and D/E cis.

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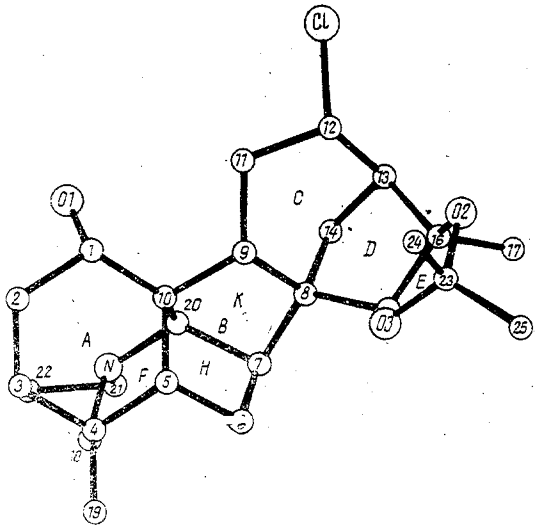


Fig. 1

TABLE 1. Bond Lengths (r, Å) and Valence angles (ω, degrees) in Structure (I)

Bond	r	Angle	ω	Angle	ω
C2 —C1	1,510 (16)	C10 —C1—C2	115.7 (8)	C14—C8 —C7	116,0 (7)
C10—C1	1,533 (12)	O1 —C1—C2	104,0 (9)	C14—C8 —C9	103,8 (7)
O1 —C1	1,351 (12)	O1 —C1—C10	114,0 (8)	C15—C8 —C7	116,6 (7)
C3 —C2	1,486 (17)	C3 —C2—C1	122,6 (1,2)	C15—C8 —C9	113,9 (7)
C4 —C3	1,538 (13)	C4 —C3—C2	115,6 (9)	C15—C8 —C14	99,7 (7)
C5 —C4	1,525 (12)	C5 —C4—C3	109.2 (7)	C8 —C9 —C10	103,8 (6)
C18—C4	1,530 (13)	C18—C4—C3	111,3 (8)	C11—C9 —C10	116,8 (7)
C19—C4	1,492 (13)	C18—C4—C5	109,1 (7)	C11—C9 —C8	111,2 (7)
C6 —C5	1,566 (12)	C19—C4—C3	107,4 (8)	C12—C11—C9	110,6 (7)
C10—C5	1,564 (11)	C19—C4—C5	111,9 (7)	C13—C12—C11	115,7 (7)
C7 —C6	1.525 (12)	C19—C4—C18	107,9 (7)	Cl —C12—C11	107,0 (6)
C17—C7	1,594 (12)	C6 —C5—C4	110,5 (7)	Cl —C12—C13	110,6 (7)
C8 —C7	1,516 (11)	C10—C5—C4	111,4 (7)	C14—C13—C12	106,5 (8)
C17—C10	1,556 (12)	C10—C5—C6	102.8 (6)	C16—C13—C12	114,3 (8)
N —C20	1,463 (9)	C7 —C6—C5	102,5 (6)	C16—C13—C14	101,3 (7)
N —C18	1,444 (10)	C17—C7—C6	104,8 (7)	C13—C14—C8	101,4 (7)
C21—C22	1,517 (14)	C8 —C7—C6	109,2 (7)	C16—C16—C13	105,1 (7)
N —C21	1,497 (12)	C8 —C7 C17	99,3 (6)	C17—C16—C13	110,0 (8)
C9 —C8	1,567 (12)	C5 —C10—C1	114,2 (7)	C17—C16—C15	114,6 (7)
C14—C8	1,562 (12)	C17—C10—C1	115,2 (7)	O2 —C16—C13	113,5 (7)
C15—C8	1,560 (12)	C17—C10—C5	98,3 (6)	O2 —C16—C15	104,3 (7)
C10—C9	1,549 (11)	C9 —C10—C1	115,3 (6)	O2 —C16—C17	109,3 (7)
C11—C9	1,548 (12)	C9 —C10—C5	109,7 (6)	C16—C15—C8	106,9 (7)
C12—C11	1,538 (13)	C9 —C10—C20	102,2 (6)	O3 —C15—C8	115,0 (7)
C13—C12	1,533 (15)	C10—C20—C7	92,4 (6)	O3 —C15—C16	105,3 (6)
Cl —C12	1,808 (8)	N —C20—C7	121.4 (6)	C25—C23—C24	112,6 (9)
C14—C13	1,537 (12)	N —C20—C10	110,4 (7)	O2 —C23—C24	110,9 (8)
C16—C13	1,539 (13)	N —C18—C4	113,5 (6)	O2 C23—C25	105,0 (8)
C16—C15	1,543 (11)	N —C21—C22	109,1 (8)	O3—C23—C24	112,5 (8)
C17—C16	1,526 (14)	C18—N —C20	117,3 (7)	O3 - C23—C25	109,7 (8)
O2 —C16	1,431 (11)	C21—N —C20	110,4 (7)	O3 —C23—O2	105,6 (7)
O3 —C15	1,397 (11)	C21—N —C18	110,8 (6)	C23 - O2 —C16	107,8 (6)
C24—C23	1,533 (14)	C9—C8—C7	102 2 (7)	C23 - O3 —C15	109,5 (7)
C25 - C23	1,546 (15)				
O2 —C23	1,435 (12)				
O3 —C23	1,409 (11)				

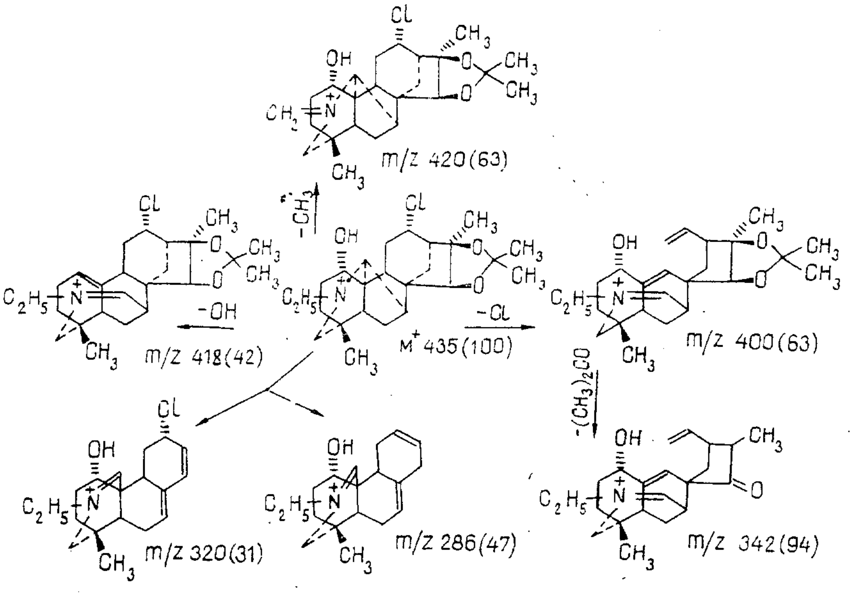
According to the literature, the position (at C1) and α-orientation of the hydroxy group and also the boat conformation of ring A favor the formation of an O—H...N H-bond in diterpene alkaloids (e.g., in akirine [2] and altaconitine [3]); however, in the structure of (I) such an intramolecular H-bond is either absent or extremely weak, as shown by the large O1...N distance of 3.14 Å and the twist-chair conformation of ring A.

Analysis of the intermolecular contacts in the crystal structure of (I) revealed no shortened contacts. The absence of intermolecular H-bonds is possibly due to the intramolecular blockage of the N atom by the hydroxy group at C1.

Bond lengths and valence angles are given in Table 1. The lengths of the ordinary C_{sp^3}—C_{sp^3} bonds range from 1.49 to 1.59 Å, but their mean value, 1.53 Å, agrees with the standard [4] and also with the values observed in related alkaloids [2, 3, 5]. The pronounced variation of the valence angles at the tetrahedral carbon atoms is due to the strain in the bridge fragments of the molecule.

Acofine — the first chlorine-containing diterpene alkaloid — has an unusual substitution of ring D, which contains a 16α-methyl group and a 15,16β-acetonide group. The biosynthesis of the substituents at C16 obviously takes place on the reduction of a 16,17-epoxy function, by analogy with the hypothesis put forward previously for dictysine-like alkaloids [6].

In the mass spectrum of acofine, in addition to peaks resulting from one-stage processes involving the ejection of hydroxyl, chlorine, and methyl radicals, we observed the peaks of ions from more far-reaching degradation formed on the splitting out of the elements of ring E and of an acetone molecule (scheme). The peak of an ion with m/z 342 corresponded to the composition $C_{22}H_{32}NO_2$.*



Mass-spectrometric fragmentation of acofine

The acetylation of acofine with acetic anhydride in the presence of pyridine gave the C1 mono-O-acetyl derivative (II) (see the formula at the beginning of the paper). The PMR spectrum of (II) contained the signal of a proton geminal to an acetoxy group at 5.05 ppm, in the form of a quadruplet (J_1 = 10 Hz; J_2 = 7 Hz). In the mass spectrum of the monoacetate, fragmentation connected with the elimination of the acetoxy radical from C1 predominated.

EXPERIMENTAL

For chromatography we used type KSK silica gel and deactivated alumina.

PMR spectra were taken on a Tesla BS-567A 100 MHz spectrometer with HMDS as internal standard, mass spectra on an MKh-1310 instrument with a direct inlet system, and IR spectra on a UR-20 spectrophotometer in KBr tablets.

Isolation of Acofine (I). Acofine (base with mp 159-160°C) was isolated by the method described in [1]. Acofine ($C_{25}H_{38}NO_3Cl$), mp 159-160°C (acetone), hydrochloride with mp 274-286°C (decomp.).

PMR spectrum, δ (ppm): 0.66 (3H, s, C4-CH_3), 0.99 (3H, t, J = 7 Hz, N—CH_2—CH_3), 1.35, 1.39, 1.44 (each 3H, s, 3 × > C—CH_3), 3.23 (1H, br.s), 4.17 (1H, q, J_1 = 10 Hz, J_2 = 7 Hz, C1β-H).

Mass spectrum, m/z (%): M 435(100), 420(63), 418(42), 400(63), 376(31), 377(33), 362(10), 342(94), 320(31), 318(15), 300(31), 286(47), 284(42), 242(42), 185(63).

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*Elementary compositions were determined by Yu. M. Mil'grom.

TABLE 2. Coordinates ($\times 10^4$) of the Nonhydrogen Atoms in the Structure of (I)

Atom	x	y	z	Atom	x	y	z
C1	—1844 (12)	6470 (5)	—0223 (4)	C16	3071 (12)	4021 (6)	0999 (4)
C2	—3013 (18)	6698 ([illegible])	—0822 (6)	C17	4863 (13)	3673 (7)	1042 (5)
C3	—3172 (11)	6155 (7)	1465 (5)	C18	—0180 (12)	6109 (6)	—1919 (4)
C4	—1652 (11)	5576 (6)	—1635 (4)	C19	—2181 (14)	4959 (7)	—2197 (5)
C5	—1099 (9)	5108 (5)	—0966 (4)	C20	1110 (11)	6083 (6)	—0728 (4)
C6	0546 (12)	4578 (5)	—1104 (4)	C21	2321 (12)	6949 (7)	—1670 (5)
C7	1895 (11)	5131 (5)	—0746 (4)	C22	1881 (16)	7767 (7)	—2073 (5)
C8	1891 (10)	4938 (5)	0037 (4)	C23	0994 (13)	2971 (7)	0955 (5)
C9	0108 (9)	5259 (5)	0270 (4)	C24	1794 (16)	2078 (6)	0846 (5)
C10	0547 (9)	5762 (5)	—0381 (4)	C25	—0752 (14)	2918 (7)	1309 (6)
C11	0195 (13)	5763 (6)	0975 (5)	O1	—1162 (11)	7237 (5)	—0035 (7)
C12	1352 (14)	5297 (6)	1501 (4)	O2	1992 (8)	3491 (4)	1422 (3)
C13	3039 (13)	4985 (7)	1205 (4)	O3	0878 (8)	3448 (4)	0324 (3)
C14	3250 (12)	5421 (6)	0483 (4)	N	0747 (9)	6559 (4)	—1377 (3)
C15	2283 (11)	3984 (6)	0257 (4)	Cl	1676 (4)	6027 (2)	2234 (1)

Acofine Monoacetate (II). A solution of 0.05 g of acofine in 2 ml of acetic anhydride and 0.2 ml of pyridine was kept at room temperature for 48 h. Then the excess of acetic anhydride was evaporated off. An aqueous solution of the residue was made alkaline with sodium carbonate and was extracted with ether. After the ether had been distilled off, the product was purified on a column of deactivated alumina. With the aid of hexane, ethereal eluates yielded 0.02 g of a homogenous pulverulent product.

PMR spectrum, δ (ppm): 0.64 (3H, s, $C4{-}CH_3$), 1.00 (3H, t, J = 7 Hz, $N{-}CH_2{-}CH_3$), 1.35, 1.40, 1.44 (each 3H, s, $3 \times > C{-}CH_3$), 2.00 (3H, s, $OCOCH_3$), 3.26 (1H, br.s), 3.85 (1H, br.s), 5.05 (1H, q, J_1 = 10 Hz, J_2 = 7 Hz, C1β–H).

Mass spectrum, m/z (%): M^+ 477(7), 462(9), 442(5), 418(100), 384(8), 382(3), 146(10). IR spectrum, ν_{max} (cm^{-1}): 1720 (C=O).

X-Ray Structural Investigation of (I). Crystals were obtained from acetone. The exposure was made on a Syntex $P2_1$ diffractometer using CuK_α radiation: a = 7.959, b = 15.438; c = 18.973 Å, d_{calc} = 1.245 g/cm^3, space group $P2_12_12_1$, Z = 4. In the calculations we used 1250 independent reflections with $I > 2\sigma$. The structure was interpreted by the direct method using the SHELXS-86 program [7]. Refinement of the structure by the least-squares method was performed by the SHELX-76 program [8] (both programs in the PC MSDOS version) in the isotropic-anisotropic approximation for all the nonhydrogen atoms. The refinement calculation included the hydrogen atoms bound to carbon atoms. The hydrogen atom of the OH group was found from a difference synthesis. The final value of the discrepancy factors were R = 0.071 and R_w = 0.071. The coordinates of the nonhydrogen atoms are given in Table 2.

LITERATURE CITED

1. M. N. Sultankhodzhaev, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 199 (1973).
2. A. A. Nishanov, B. Tashkhodzhaev, I. M. Yusupova, and M. N. Sultankhodzhaev, Khim. Pr. Soedin., 534 (1992).
3. N. Batbayar, D. Batsurén, B. Tashkhodzhaev, M. I. Yusupova, and M. N. Sultankhodzhaev, Khim. Pr. Soedin., No. 1, 60 (1993).
4. F. H. Allen, O. Kennard, D. G. Watson, L. Bramer, A. G. Orpen, and R. Taylor, J. Chem. Soc., Perkin Trans. II, No. 12, S1-S19 (1987).
5. B. Tashkhodzhaev, Khim. Pr. Soedin., 231 (1982).
6. M. N. Benn, F. Oranga, and J. F. Richardson, Heterocycles, 26, 2331 (1987).
7. G. M. Sheldrick, SHELXS-86 Program for Crystal Structure Determination, Gottingen, GFR.
8. G. M. Sheldrick, SHELX-76 Program for Crystal Structure Determination, Cambridge University Press, Cambridge, UK (1976).