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ALKALOIDS OF Aconitum kirinense.

STRUCTURE OF AKIRINE

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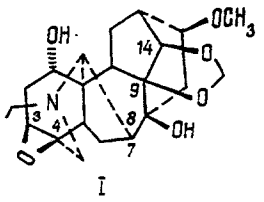
The known alkaloid lepenine and the new diterpene alkaloid akirine have been isolated from the epigeal part of Aconitum kirinense Nakai. To establish the structure of akirine, its spectral characteristics have been studied and an x-ray structural analysis has been made. It is 1α,8β-dihydroxy-16β-methoxy-9β,14β-methylenedioxy-3β,4β-epoxy-N-ethylaconitane – the first diterpene alkaloid with a lycoctonine skeleton containing a 9,14-methylenedioxy group and a β-oriented substituent at C14.

Continuing the separation of the total alkaloids of the epigeal part of Aconitum kirinense Nakai [1], we have isolated lepenine [2] and a new alkaloid with the composition $C_{22}H_{31}NO_6$, which has been called akirine (I). Its IR spectrum contained absorption bands of hydroxy groups at 3515 and 3200 cm^{-1} and of ether bonds at 1110 cm^{-1}. The PMR spectrum showed the signals of N-ethyl, methoxy, and methylenedioxy groups. The mass spectrum of (I) was characteristic for alkaloids with a lycoctonine skeleton containing a hydroxy group at C1 and a 3,4-epoxy function, and was similar to that of excelsine [3, 4].

In all previously known alkaloids with a methylenedioxy group it is located in the 7, 8 position. The mass spectra of these alkaloids have a number od diagnostic features due to the presence of the methylenedioxy group – in particular, the peak of the $(M - 30)^+$ ion [5]. In the mass spectrum of akirine the peak of the $(M - 30)^+$ ion was observed at a level far below that of the $(M - 31)^+$ ion and, consequently, is not evidence in favor of the 7, 8 position.



I

An x-ray structural investigation of akirine showed that this alkaloid had the structure (I) and is the first alkaloid with a lycoctonine skeleton containing a 9,14-methylenedioxy group and a β-oriented substitutent at C14. Thus, alkaloid (I) was 1α,8β-dihydroxy-16β-methoxy-9β,14β-methylenedioxy-3β,4β-epoxy-N-ethylaconitane. The biosynthesis of such alkaloids apparently takes place through the 14-dehydro derivative, the enzymatic reduction of which can give not only the usual α- but also the β- epimer, with the subsequent formation of a methylenedioxy group.

The spatial structure of the (I) molecule as a projection on the plane of the three atoms C1C4C9 is shown in Fig. 1. The molecule has a rigid bridge structure with the following orientations of the substituents: α-hydroxy group at C1, β-3,4-epoxy group, β-3,4-methylenedioxy group, β-hydroxy group at C16. Conformations of the main rings: six-membered

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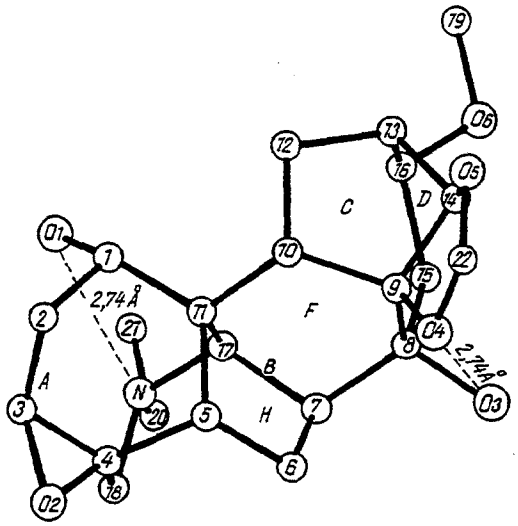

Fig. 1. Spatial structure of the akirine molecule.

ring A (the C1–C5, C11 atoms) – boat, with an accuracy of ±0.06 Å and deviations of the C2 and C5 atoms from the plane of the other four by 0.73 and 0.76 Å, respectively; the seven-membered ring B (C5–C11) – also boat (±0.05) Å with deviations of the C5, C6 and C9 atoms by 1.30, 1.51, and 0.46 Å, respectively; the five-membered ring C (C9, C10, C12–C14) – envelope (±0.005 Å), with deviation of the C14 atom by 0.63 Å; ring D (C8, C9, C13–C16) – boat (±0.003 Å) but flattened at C15 (deviation of the C15 atom (0.22 Å) less than at C14 (0.92 Å). The rings formed with the participation of the C17 and C18 carbon atoms and the N atom have the following conformations: ring E (C4, C5, C11, C17, C18, N) – chair (± 0.05 Å) with deviations of the C11 and C18 atoms (0.94 and –0.67 Å, respectively; ring F (C7–C11, C17) – chair (±0.05 Å with deviations of the C9 and C17 atoms (–0.46 and 0.84 Å, respectively). The five-membered rings H (C5–C7, C11, C17) and the closed methylenedioxy group (C9, O4, C22, O5, C14) have a conformation close to the half-chair type, the two atoms deviating on either side from the plane of the other three being, in the first case, C11 (–0.52 Å) and C19 (0.36 Å), and, in the second case, O4 (–0.43 Å) and O5 (0.51 Å). The linkages of the main rings are as follows: A/B – trans; A/F –cis; B/C – cis; B/D – cis; and B/E – cis. The ring of the methylenedioxy group is cis-linked with ring C.

The conformation of ring A in (I) is apparently stabilized by an intramolecular H-bond, O1–H...N (O...N distance 2.78 Å). This follows from the fact that literature information on the stereochemistry of alkaloids of the lycoctine series (for example, [6-9]) shows that only when an OH group is present in the C1 position is a boat conformation of ring A observed, while in other cases it has the chair conformation.

It is possible that the H atom of the second hydroxy group, O3H, also participates in the formation of an intramolecular H-bond (O3...O4 distance 2.74 Å) since the intermolecular contacts do not reveal a shortening of intermolecular distances due to an H-bond with the participation of O3.

The bond lengths and valence angles are given in Table 1, their values having been determined with errors of not more than 0.018 Å and 1.2°, respectively. The lengths of the ordinary C_{sp^3}–C_{sp^3} bonds range between 1.50 and 1.58 Å, and those of the C–O bonds between 1.40 and 1.47, which agree to within the 3σ limits with those generally accepted [10]. The appreciable shortening of the C3–C4 bond is due to the presence of the epoxy group. The considerable variation in the valence angles at the tetrahedral carbon atoms is connected with the strain in the bridge fragments of the molecule (Table 1).

EXPERIMENTAL

Mass spectra were taken on a MKh-1310 instrument with a system for direct introduction into the ion source; IR spectra on a UR-20 spectrometer (KBr); and PMR spectra on a Tesla BS-567 A instrument (100 Mhz, δ scale, 0 – HMDS). For chromatography we used type KSKG silica gel with a grain size of 0.055-0.125 mm.

TABLE 1. Bond Lengths (r, Å) and Valence Angles (ω, degrees)

Distance	r	Angle	ω	Angle	ω
C1—C2	1,57	C11—C1—C2	109	O4—C9—C10	112
C1—C11	1,58	O1—C1—C2	109	O4—C9—C8	115
C1—O1	1,41	O1—C1—C11	113	O4—C9—C14	102
C2—C3	1,50	C3—C2—C1	106	C9—C10—11	118
C3—C4	1,48	C2—C3—C4	113	C9—C10—C12	103
C4—C5	1,54	C2—C3—O2	124	C11—C10—C12	114
C3—O2	1,44	O2—C3—C4	69	C1—C11—C5	112
C4—O2	1,47	C2—C4—C3	59	C1—C11—C10	107
C5—C6	1,53	C3—C4—C5	113	C1—C11—C17	118
C5—C11	1,58	C18—C4—C3	118	C5—C11—C10	113
C6—C7	1,58	C18—C4—C5	115	C5—C11—C17	95
C7—C8	1,53	O2—C4—C5	114	C10—C11—C17	110
C8—C9	1,54	O2—C4—C18	125	C10—C12—C13	105
C8—C15	1,56	C4—C5—C6	112	C12—C13—C14	112
C8—O3	1,42	C4—C5—C11	102	C12—C13—C16	112
C9—C10	1,57	C6—C5—C11	106	C14—C13—C16	104
C9—C14	1,54	C5—C6—C7	103	C13—C14—C9	100
C9—O4	1,42	C6—C7—C8	111	O5—C14—C13	116
C10—C11	1,52	C6—C7—C17	103	O5—C14—C9	100
C10—C12	1,53	C8—C7—C17	112	C16—C15—C8	121
C11—C17	1,52	C7—C8—C9	109	C4—C18—N	113
C12—C13	1,50	C7—C8—C15	112	C7—C17—N	113
C13—C14	1,50	C7—C8—O3	111	C7—C17—C11	102
C13—C16	1,54	C15—C8—C9	110	C11—C17—N	113
C14—O5	1,46	O3—C8—C9	111	C21—C20—N	114
C15—C16	1,56	O3 C8—C15	104	O4—C22—O5	110
C16—O6	1,44	C10—C9—C8	113	C3—O2—C4	61
C19—O6	1,43	C14—C9—C8	108	C22—O4—C9	106
C18—N	1,49	C10—C9—C14	105	C14—O5—C22	106
C17—N	1,45			C16—O6— C19	112
C20—N	1,47				
C20—C21	1,53				

TABLE 2. Coordinates (×10⁴) of the Nonhydrogen Atoms in the Structure of (I).

Atom	x	y	z	Atom	x	y	z
C1	—2016 (16)	3618 (9)	5475 (6)	C16	3755 (15)	3794 (10)	6583 (7)
C2	—3729 (18)	4018 (12)	5291 (7)	C17	0334 (15)	5143 (9)	5513 (6)
C3	—3528 (16)	5023 (10)	4842 (6)	C18	—1117 (17)	6325 (9)	4697 (6)
C4	—2479 (15)	5936 (8)	5100 (5)	C19	4966 (32)	2338 (22)	6884 (18)
C5	—2114 (17)	5797 (9)	5821 (6)	C20	1550 (18)	5744 (14)	4480 (6)
C6	—0995 (16)	6737 (9)	6062 (5)	C21	2537 (25)	4713 (20)	4261 (13)
C7	0588 (15)	6173 (9)	5952 (5)	C22	—1083 (23)	4904 (13)	7913 (7)
C8	1413 (13)	5303 (8)	6503 (5)	O1	—1227 (11)	3223 (6)	4918 (4)
C9	0211 (15)	5043 (9)	6960 (5)	O2	—4148 (11)	6163 (7)	4961 (4)
C10	—0744 (15)	4218 (9)	6505 (4)	O3	1856 (11)	6790 (6)	6963 (4)
C11	—1132 (14)	4636 (9)	5830 (5)	O4	—0790 (10)	5660 (7)	7392 (3)
C12	0248 (17)	3054 (9)	6524 (7)	O5	—0131 (11)	3025 (6)	7860 (3)
C13	1630 (18)	3314 (9)	6956 (6)	O6	4522 (11)	3590 (8)	6911 (5)
C14	1134 (17)	4239 (9)	7418 (5)	N	0078 (12)	5427 (7)	4810 (4)
C15	2991 (18)	5131 (11)	6503 (7)				

Separation. The total ether-soluble alkaloids from the epigeal part of Aconitum kirinense [1] were separated into phenolic and nonphenolic fractions. For this purpose, they were dissolved in 5% H_2SO_4, and, with cooling, the solution was made alkaline with sodium carbonate and was extracted with ether and then with chloroform. The etheral solution was treated with a 1% solution of caustic soda and was then washed with water and dried over sodium sulfate. Distillation of the ether left 2.24 g of nonphenolic fraction. The alkaline solution was saturated with ammonium chloride and extracted with ether and then with chloroform. This gave 1.39 g of ether-soluble and 2.46 g of chloroform-soluble phenolic fractions.

Column Chromatography of the Nonphenolic Ether-Soluble Fraction. The ether-soluble fraction (2.24 g) was chromatographed on a column of silica gel with elution by benzene to which acetone was gradually added. Fractions 10-13 from elution with benzene—acetone (10:1) yielded 0.1 g of technical 8-acetylexcelsine. Lepenine was isolated from fractions 30-44 on elution with benzene—acetone (1:1). Yield 0.45 g.

Column Chromatography of the Phenolic Ether-Soluble Fraction. The ether-soluble fraction (1.59 g) was chromatographed on a column of silica gel. By treatment with acetone, fractions 1-2 from elution with benzene—acetone (1:1) yielded 0.05 g of akirine.

Akirine (I) $C_{22}H_{31}NO_6$, (M^+ 405.2170, HRMS), mp 214-217°C (acetone). IR spectrum, ν_{max}^{KBr} (cm^{-1}): 3515, 3200 (OH), 1110 (C–O–C). Mass spectrum, m/z (%): M^+ 405(100), 390(60), 388(43), 377(11), 375(15), 374(60, 362(15), 360(7), 358(6), 356(4), 350(9), 346(6), 344(5), 334(9), 332(6), 330(5), 328(5), 319(11), 316(5), 303(14), 235(27), 218(8), 208(8), 207(7), 206(8), 190(6). PMR spectrum: (100 MHz, $CDCl_3$); 1.02 (3H, t, J = 7.5 Hz, N–C_2H_5), 3.24 (3H, s, OCH_3), 5.24, 5.03 (1H each, –O–CH_2–O–), 3.94 (1H, s), 3.46 (1H, s).

X-Ray Structural Investigation. The cell parameters and the intensities of 1544 reflections ($I > 2\sigma$) were measured on a Syntex $P2_1$ diffractometer (Cu-K_α radiation): a = 8.470(2), b = 11.56(5), c = 20.751(7) Å, V = 2033.2(1, 2) Å, $d_{calc.}$ = 1.325 g/cm³, space group $P2_12_12_1$, Z = 4. The structure was interpreted by the direct method, using the SHELXS-86 program [11]. Refinement was carried out by the full-matrix method of least squares in the isotropic-anisotropic approximation (SHELX-76 program [12]), R = 0.071, R_w = 0.074. The hydrogen atoms were placed in their calculated positions. The coordinates of the nonhydrogen atoms are given in Table 2.

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