ZERAVSHANISINE – A NEW ALKALOID FROM Aconitum zeravschanicum

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UDC 547.944/945+548.737

The new diterpene alkaloid zeravshanisine has been isolated from the epigeal part of Aconitum zeravschanicum Steinb.. Its structure has been established by spectral methods and by x-ray structural analysis as 2-acetyl-13-benzoyl-14-hydroxyhetisine.

Continuing a study of the alkaloids of Aconitum zeravschanicum Steinb., from the epigeal part of the plant collected in the budding–incipient flowering stage in the environs of the village of Djhirgatal' (Tadzhikistan), in addition to heteratisine [1] and atisine, isoatisine, nominine, and atisine chloride [2], detected previously, we have isolated a new base with the composition $C_{29}H_{33}NO_6$ (I) which we have called zeravshanisine.

Zeravshanisine is an amorphous base giving a crystalline perchlorate with mp 287-289°C (decomp., ethanol). The IR spectrum of (I) contained absorption bands at (cm^{-1}) 3620-3400 (OH group); 3080, 1660, 890 (terminal methylene group); 1730, 1280 (ester grouping), and 1605, 1590 (aromatic ring). According to its PMR spectrum taken in $CDCl_3$ solution with HMDS as internal standard (Table 1), zeravshanisine contained tertiary methyl, acetyl, terminal methylene, and benzoyl groups. The mass spectrum of the base contained the peaks of ions with m/z 491 (M^+), 476, 474, 448, 432, 386 (100%), 370, and 105.

The IR, PMR, and mass spectra of (I) enabled it to be assigned to the C_{20}-diterpene alkaloids of the hetesine type.

The alkaline hydrolysis of (I) gave an amino alcohol (II) with mp 234-236°C (ether–acetone), M^+ 345, and benzoic and acetic acids.

A comparison of the PMR spectra of zeravshanisine with that of Guan-fu base Z (III), isolated from Aconitum koreanum [3] showed that bases (I) and (III) contained one and the same amino acid (II). The spectrum of (III) contained one-proton signals at 5.13, 4.22, and 4.04 ppm assigned, respectively, to the C2-βH, C11-βH, and C13-αH protons. The analogous signals in the spectrum of (I) appeared at δ 5.15, 4.34, and 5.46 ppm, respectively, which showed the presence of the acetoxy group at C2 and of the benzoloxy group at C13.

TABLE 1. Details of the PMR Spectra of Zeravshanisine (I) and of Guan-fu Base (III) ($CDCl_3$, δ scale, ppm)

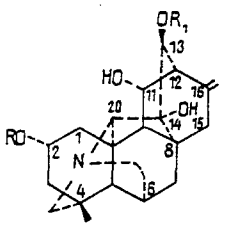
Protons	I	III
C2–βH	5,15 (m)	5,13 (m)
C11–βH	4,34 (d, J=9 Hz)	4,22 (d, J=8,7Hz)
C13–αH	5,46 (br.s)	4,04 (br.s)
C17–H_a	4,88 (br.s); 4,70 (br.s)	4,86 (br.s); 4,68 ( )
C18–H_3	0,90 (s)	1,01 (s)
C19–H_a	2,67 (d, J=12 Hz)	2,95 (d, J=12,2Hz)
C19–H_b	2,40 (d, J=12 Hz)	2,52 (d, J=12,2Hz)
C20–H	3,23 (s)	3,53 (s)
$(CH_3)_2CHCO$	—	1,16 (d, J=6,8Hz)
CH_3CO	1,13 (s)	—
Ar–H	7,35–8,10 (m)	—

Notes. s) singlet; br. s) broadened singlet; d) doublet; m) multiplet.

Institute of Chemistry of Plant Substances, Uzbekistan Republic Academy of Sciences, Tashkent. Institute of Organometallic Compounds, Russian Academy of Sciences, Moscow. Translated from Khimiya Prirodnykh Soedinenii, Nos. 3,4, pp. 375-382, May-August, 1992. Original article submitted September 30, 1991.

0009-3130/92/0304-0329$12.50

On the basis of the facts given above it may be concluded that zeravshanisine is 2-acetyl-13-benzoyl-14-hydroxyhetesine



I. $R=CH_3CO$; $R_1=C_6H_5CO$
II. $R=R_1=H$
III. $R=(CH_3)_2CHCO$; $R_1=H$

Because of the absence of a sample of compound (III) we were unable to perform a chemical identification by comparing their alcohols. For this reason, in order to establish the structure of the diterpene alkaloid zeravshanisine reliably we have carried out an x-ray structural analysis of its perchlorate.

The structure of the cation of (I) in projection on a plane with the least overlapping of the atoms is shown in Fig. 1. As can be seen from Fig. 1, the x-ray structural investigation confirmed the structure and stereochemistry proposed above for zeravshanisine. The cation of (I) contained a rigid three-dimensional hetisine skeleton of nine rings with the following substituents: acetoxy (2α), methyl on tertiary carbon (4β), terminal methylene (16), benzoyloxy (13β), and two OH groups (11α and 14α). The stereochemistry – the conformations and linkages of the main rings of the hetisine nucleus of the cation of (I) – agreed completely with those observed in talatisine (IV) [4]: the six-membered rings A and B had the chair conformation (ring B slightly distorted), six-membered rings D, and E distorted boat conformation (the distortions being connected with the closure of ring F), the five-membered ring F, G, and H envelope conformations, and heterocycle K the boat conformation; ring linkages: A/B-trans, A/H-cis, B/C-trans, B/H-cis, B/K-trans, C/F-cis, D/F-cis, F/G-cis, and F/K-cis.

Table 2 gives the numerical characteristics of the conformation of the rings in comparison with those calculated for talatisine. In the benzoyloxy fragment the angle between the benzene ring and the ester group is 21.3°, which favors conjugation between these groups. The syn direction of the benzoyloxy and acetoxy (at C2) groups and also the almost perpendicularity of their planes (79.8°) (see Fig. 1) confirms the possibility of effective screening of the methyl group ($C_{29}H_3$) by the π-electron system of the benzene ring. Consequently, the appearance of a signal of methyl protons of an acetoxy groups in the spectra of (I) in

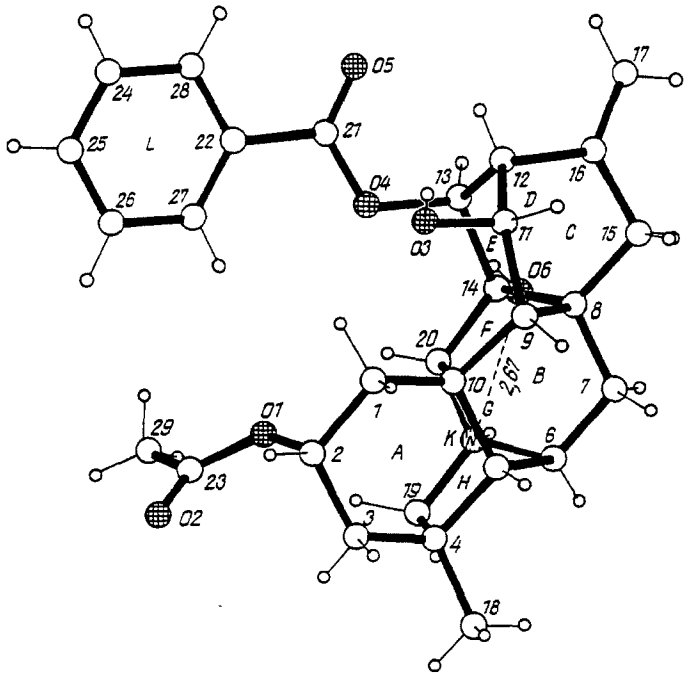


Fig. 1. Structure of the zeravshanisine molecule.

TABLE 2. Main Planes of the Fragments and Deviations of the Atoms from these Planes (δ, Å) in Zeravshanisine (I) and Talatisine (IV) [4].

Ring	Atom	δ_I	δ_{IV}	Ring	Atom	δ_I	δ_{IV}
	C1	−0,011	−0,027		C5	−0,070	−0,074
	C2	0,011	0,027		C7	0,071	0,077
A	C4	−0,010	−0,025	B	C8	−0,077	−0,082
	C5	0,010	0,025		C10	0,076	0,079
	C3*	−0,600	−0,531		C6*	0,646	0,615
	C10*	0,614	0,625		C9*	−0,857	−0,876
	C9	0,124	0,113		C9	−0,062	−0,068
	C11	−0,128	−0,115		C11	0,058	0,063
C	C13	0,132	0,120	D	C16	−0,063	−0,058
	C14	−0,127	−0,117		C15	0,062	0,068
	C8*	0,618	0,648		C8*	0,916	0,876
	C12*	0,692	0,692		C12*	0,618	0,717
	C16	0,040			C9	0,015	0,031
	C15	−0,037			C10	−0,022	−0,046
E	C13	0,039		F	C15	−0,015	−0,031
	C14	−0,041			C17	0,046	0,022
	C8*	0,610			C8*	0,792	0,784
	C12*	0,810					
	C5	0,001	0,006		C4	0,014	0,004
	C10	−0,001	−0,008		C5	−0,010	−0,003
G	C17	0,001	0,008	H	N	0,011	0,003
	N	−0,011	−0,006		C18	−0,015	−0,004
	C6*	0,893	0,876		C6*	0,872	0,866
	C4	0,012			C22	0,001	
	C10	−0,012			C23	−0,012	
K	C18	−0,013		L	C24	0,003	
	C17	0,013			C25	−0,003	
	C5*	0,817			C26	0,003	
	N*	0,801			C27	−0,002	

*Atoms not included in the calculation of the equation of the plane.

in a stronger field by 1 ppm than for known alkaloids of the hetisine type [5, 6] becomes completely explicable.

Bond lengths and valence angles are given in Table 3. The lengths of the ordinary C_{sp^3}–C_{sp^3} bonds vary within the fairly wide limits of 1.490-1.582 Å, but they agree with the generally adopted value of 1.54 Å to within 3σ. The divergences in the lengths of the other types of C–C bonds and of hetero bonds of the N–C, C–O, and C=O types are small, and their values are close to the standard values [7]. A considerable variation of the angles from 93.0 to 117.4° at the tetrahedral carbon atoms is connected with the strain existing in the bridge fragments of the molecule [4, 8]. The bond lengths and valence angles in the plane benzene ring are the usual ones. The mutual positions of the hydroxy group at C-14 and the N atom would be favorable for the formation of an intramolecular H-bond of the O–H... N distance is 2.67 Å) in base I (Fig. 1). However, the H atoms found experimentally show that on the protonation of the N atom in the perchlorate hydrogen bonds are formed differently. Figure 2 shows the packing of the perchlorate of (I) and of all types of H-bonds realized in which all three active H atoms participate.

EXPERIMENTAL

The homogeneity of the substances was checked by chromatography in a thin layer of type KSK silica gel in the chloroform–methanol (20:1) and chloroform–ethanol (9:1) systems and in a thin layer of alumina of "for chromatography" grade in the chloroform–methanol (50:1), chloroform–methanol (4:1), benzene–ethanol (9:1), and ether–hexane (3:1) systems. IR spectra were taken on a UR-20 instrument, PMR spectra on a BS-567 A NMR instrument (Tesla, Czechoslovakia) at a frequency of 100 MHz, and mass spectra on a MKh-130 instrument fitted with a system for direct introduction into the ion source.

TABLE 3. Interatomic Distances (r, Å) and Valence Angles (ω, degrees)

Distance	r	Distance	r	Angle	ω	Angle	ω	Angle	ω
O1—C2	1,480(5)	C8—C14	1,556(5)	C28—O1—C2	117,1(3)	C14—C8—C9	98,4(3)	C20—C14—O6	111,4(3)
O1—C28	1,334(5)	C9—C10	1,576(6)	C21—O4—C13	114,5(3)	C14—C8—C15	113,1(3)	C20—C14—C8	100,9(3)
O2—C28	1,207(6)	C9—C11	1,548(6)	C20—N—C6	102,2(3)	C11—C9—C8	106,8(3)	C20—C14—C13	113,6(3)
O3—C11	1,435(6)	C10—C20	1,563(6)	C19—N—C6	102,7(3)	C10—C9—C8	102,3(3)	C10—C20—N	103,5(3)
O4—C13	1,452(5)	C11—C12	1,532(7)	C19—N—C20	110,1(3)	C11—C9—C10	121,1(3)	C14—C20—N	107,3(3)
O4—C21	1,350(5)	C12—C16	1,516(7)	C10—C1—C2	115,3(3)	C9—C10—C1	117,8(3)	C14—C20—C10	106,3(3)
O5—C21	1,189(6)	C12—C13	1,529(6)	C1—C2—O1	107,9(3)	C5—C10—C1	112,3(3)	C4—C18—N	104,5(3)
O6—C14	1,426(4)	C15—C16	1,494(7)	C3—C2—O1	109,8(3)	C9—C10—C5	105,7(3)	O5—C21—O4	123,5(4)
N—C6	1,522(4)	C16—C17	1,338(7)	C3—C2—C1	114,2(3)	C20—C10—C1	115,8(3)	C22—C21—O4	111,8(4)
N—C20	1,510(6)	C13—C14	1,547(5)	C4—C3—C2	116,7(3)	C20—C10—C5	100,6(3)	C22—C21—O5	124,7(4)
N—C19	1,504(5)	C14—C20	1,517(5)	C5—C4—C3	110,8(3)	C20—C10—C9	102,7(3)	C23—C22—C21	117,2(4)
C1—C2	1,515(6)	C21—C22	1,489(6)	C19—C4—C3	115,1(3)	C9—C11—O3	111,5(4)	C27—C22—C21	122,9(4)
C1—C10	1,525(5)	C22—C23	1,399(7)	C19—C4—C5	100,0(3)	C12—C11—O3	111,9(4)	C27—C22—C23	119,8(4)
C2—C3	1,493(6)	C22—C27	1,360(7)	C18—C4—C3	108,3(3)	C12—C11—C9	110,0(4)	C24—C23—C22	119,2(5)
C3—C4	1,528(6)	C23—C24	1,374(8)	C18—C4—C5	112,5(3)	C16—C12—C11	100,7(4)	C25—C24—C23	120,4(6)
C4—C5	1,542(6)	C24—C25	1,362(9)	C19—C4—C18	110,0(3)	C13—C12—C11	111,2(4)	C26—C25—C24	120,7(6)
C4—C19	1,561(5)	C25—C26	1,373(8)	C6—C5—C4	102,7(3)	C13—C12—C16	103,6(4)	C27—C26—C25	119,3(5)
C4—C18	1,530(6)	C26—C27	1,387(7)	C10—C5—C4	111,4(3)	C15—C16—C12	111,3(4)	C26—C27—C22	120,5(5)
C5—C6	1,523(5)	C28—C29	1,489(6)	C10—C5—C6	101,4(3)	C17—C16—C12	122,9(5)	O2—C28—O1	123,9(4)
C5—C10	1,582(5)	C1—O7	1,403(3)	C5—C6—N	93,0(3)	C17—C16—C15	125,5(5)	C29—C28—O1	112,8(3)
C6—C7	1,490(6)	C1—O8	1,411(4)	C7—C6—N	111,3(3)	C16—C15—C8	109,2(4)	C29—C28—O2	123,3(4)
C7—C8	1,525(6)	C1—O9	1,419(4)	C7—C6—C5	117,4(3)	C12—C13—O4	111,5(3)	O8—C1—O7	108,9(2)
C8—C9	1,534(6)	C1—O10	1,405(5)	C8—C7—C6	111,2(3)	C14—C13—O4	110,4(3)	O9—C1—O8	110,7(2)
C8—C15	1,528(5)			C9—C8—C7	110,4(3)	C14—C13—C12	109,7(3)	O9—C1—O7	113,5(2)
				C15—C8—C7	110,7(3)	C8—C14—O6	109,9(3)	O10—C1—O7	108,9(3)
				C15—C8—C9	114,3(3)	C13—C14—O6	111,2(3)	O10—C1—O8	107,3(3)
				C14—C8—C7	109,2(3)	C13—C14—C8	109,2(3)	O10—C1—O9	108,3(3)

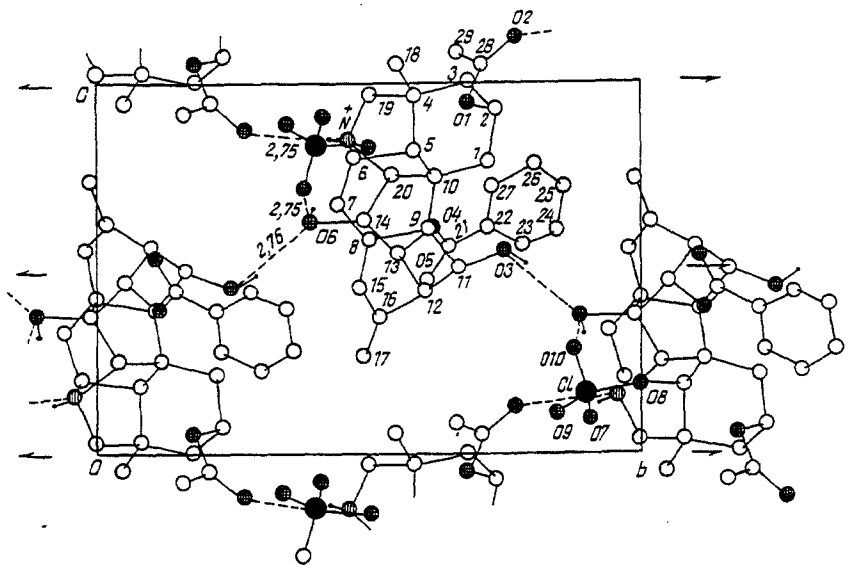


Fig. 2. Packing of the molecules of zeravshanisine perchlorate.

Isolation of the Alkaloids. The comminuted dry epigeal part of the plant (2.2 kg) was wetted with a 5% solution of sodiumc arbonate and was left at room temperature for 2 h. Then chloroform was added and the mixture was left to the following day. A total of ten extractions was carried out. The combined extracts were concentrated and were treated with 5% H_2SO_4. The acid solution was filtered and washed with ether, and it was then made alkaline to pH 8-9 with cooling and was extracted with chloroform. Caustic potash was then added to the alkaline aqueous solution to pH > 12, and it was extracted with chloroform. After the extract had been dried with sodium sulfate and the solvent had been distilled off, 17.1 g of a mixture of alkaloids at pH 8-9 (part A) and 1.5 g of a mixture at pH 12 (part B) were obtained. The plant material (after the elimination of traces of chloroform) was covered with ethanol and the mixture was left to the following day.

Five extractions were made. The combined extracts were concentrated to 300 ml, and 300 ml of acetone was added. The resulting precipitate was separated off and washed with acetone. The acetone solution was evaporated to dryness. The residue was treated with 5% H_2SO_4, and the acetone solution was filtered, washed with ether, made alkaline with caustic potash to pH 12 with cooling, and extracted with chloroform. After the chloroform extract had been dried with sodium sulfate and the solvent had been distilled off, 1.24 g of a mixture of alkaloids (part C) was obtained.

Part A was deposited on a column of silica gel (1:20). The substances were eluted with chloroform–ethanol in ratios of (100:1) (fractions 1-23), (50:1) (fractions 24-37), and (10:1) (fractions 38-60), and then with 5% H_2SO_4 (fractions 107-113), the volume of each fraction being 200 ml. When fractions 39-60 were treated with acetone, 1.75 g of heteratisine separated out. The minimum amount of ethanol was added to the mother solution and it was acidified with a 10% alcoholic solution of $HClO_4$, giving 0.47 g of heteratisine perchlorate. On concentration of the mother solution, 0.64 g of zeravshanisine perchlorate separated out. The acidification of fractions 61-67 with a 10% alcoholic solution of $HClO_4$ led to the separation of 0.31 g of heteratisine perchlorate.

Fractions 107-113 were alkalinized with sodium carbonate to pH 9-10 and were extracted with chloroform. After the extract had been dried with sodium sulfate and the solvent had been distilled off, 2.5 g of a mixture of alkaloids was obtained which was chromatographed on a column of alumina (1:30). The substances were eluted with benzene–ethanol in ratios of 100:1 (fractions 1-13) and 10:1 (fractions 14-25), the volume of each fraction being 30 ml. On treatment with acetone–ethanol (2:1), fraction yielded 0.1 g of nominine. On treatment with acetone, fractions 15-19 gave 0.035 g of a base with mm 357.

On treatment with chloroform, part C yielded 0.32 g of atisine chloride. The mother solution after the separation of the atisine chloride was chromatographed on a column of alumina (1:70). The substances were eluted with chloroform (fractions 1-12) and with chloroform–methanol (100:1) (fractions 27-70). By treatment with chloroform, 0.13 g of atisine chloride was isolated. Fractions 3-6 (0.36 g) were rechromatgraphed on a column of

TABLE 4. Coordinates (×10⁴) of the Nonhydrogen Atoms in the Structure of Zeravshanisine

Atom	x	y	z	Atom	x	y	z
C1	0716 (5)	7171 (3)	7893 (4)	C19	0931 (4)	5009 (3)	9668 (3)
C2	0474 (4)	7252 (3)	9259 (4)	C20	0094 (4)	5364 (2)	7547 (3)
C3	1635 (4)	6794 (3)	10041 (4)	C21	−3202 (5)	6415 (3)	5485 (4)
C4	2134 (4)	5797 (3)	9655 (4)	C22	−3981 (4)	7178 (3)	6143 (4)
C5	2514 (4)	5781 (3)	8285 (4)	C23	−4950 (5)	7754 (4)	5466 (5)
C6	2425 (4)	4721 (3)	7971 (3)	C24	−5686 (6)	8471 (4)	6053 (7)
C7	2571 (4)	4446 (3)	6659 (4)	C25	−5435 (6)	8625 (4)	7277 (7)
C8	1555 (4)	5035 (3)	5831 (4)	C26	−4518 (6)	8061 (4)	7948 (5)
C9	1749 (4)	6107 (3)	6093 (4)	C27	−3771 (5)	7337 (3)	7365 (5)
C10	1226 (4)	6193 (3)	7456 (4)	C28	−1603 (4)	7094 (3)	10543 (4)
C11	0957 (5)	6649 (3)	5035 (4)	C29	−2968 (5)	6559 (3)	10771 (5)
C12	−0199 (5)	6403 (3)	4430 (4)	N	0853 (3)	4643 (4)	8371 (2)
C13	−1078 (6)	5474 (3)	5394 (3)	O1	−0954 (3)	6816 (2)	9520 (2)
C14	−0034 (5)	4924 (3)	6275 (3)	O2	−1137 (3)	7713 (2)	11247 (3)
C15	1731 (4)	4758 (3)	4487 (4)	O3	0349 (4)	7534 (2)	5452 (3)
C16	0536 (4)	5204 (4)	3733 (4)	O4	−1986 (3)	6125 (2)	6076 (2)
C17	0046 (8)	4890 (4)	2641 (4)	O5	−3578 (5)	6106 (3)	4493 (3)
C18	3418 (5)	5503 (4)	10480 (4)	O6	−0410 (3)	3935 (2)	6335 (2)
			Atom				
Cl	3477 (1)	9035 (1)	1622 (1)	O9	4571 (3)	8461 (3)	1091 (4)
O7	2192 (3)	9090 (3)	0905 (4)	O10	3151 (5)	8650 (4)	2774 (4)
O8	4016 (4)	9969 (3)	1822 (5)				

silica gel (1:50). The substances were eluted with chloroform (fractions 1-3) and with chloroform–methanol (100:1) (fractions 4-11) and 10:1 (fractions 12-30), the volume of each fraction being 30 ml. By treatment with acetone, fractions 16-26 yielded 0.055 g of isoatisine.

Saponification of Zeravshanisine. A mixture of 0.2 g of zeravshanisine and 10 ml of 5% methanolic KOH was boiled for 1 h. The solvent was evaporated off and the residue was diluted with water. The aqueous solution was extracted with chloroform. The combined chloroform extracts were dried with sodium sulfate. The residue after the solvent had been distilled off was treated with ether–acetone (4:1), and 0.11 g of the amino alcohol of zeravshanisine separated out. The alkaline aqueous solution was treated with 5% H_2SO_4 to pH 3-4 and was extracted with ether. The ethereal extracts were combined, and, after the addition of NH_4OH, concentrated. Paper chromatography showed the presence of acetic and benzoic acids.

The x-ray structural experiment was conducted on a Hilger–Watts four-circle diffractometer (λ, MoK_α $\theta/2\theta$ scanning, $\theta < 26°$): $a = 9.188(2)$, $h = 13.977(4)$, $c = 10.900(4)$ Å, $\beta = 91.26°(1)$; $d_{calc} = 1/.348$ g/cm³, space group $P2_1$, $z = 2$. In the interpretation and refinement of the structure we used 2686 reflections with $I \geq 2\sigma$. The structure was interpreted by the direct method using the MULTAN program and was refined by the full-matrix method of least squares in the anisotropic approximation for all the nonhydrogen atoms. The coordinates of the H atoms at the carbon atoms were calculated theoretically and those at the heteroatoms O and N were found in a difference electron density synthesis. All the H atoms were refined isotropically. The final values of the divergence factors were $R = 0.040$ and $R_w = 0.041$. All the calculations were made on an Eclipse S/200 computer by the INEXTL program [9]. The coordinates of the basis atoms of the structure of the perchlorate of I are given in Table 4.

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