Berberis ALKALOIDS.

XXI. INTEBRINE – A NEW N-BENZYLISOQUINOLINE ALKALOID FROM Berberis integerrima

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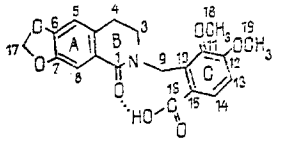
A new N-benzylisoquinoline alkaloid – intebrine, with the composition $C_{20}H_{19}NO_7$ – has been isolated from Berberis integerrima Bunge. The spatial structure of intebrine has been reliably determined by the x-ray structural method (diffractometer, CuK_α radiation, 972 reflections, direct method, R = 0.048). Its chemical behavior, spectral characteristics, and mass-spectrometric fragmentation under electrom impact have been studied.

The isolation of alkaloids of the isoquinoline series from B. integerrima Bunge has been reported previously [1-3].

Continuing a study of the alkaloids of this plant, we extracted the leaves gathered in the phase of mass flowring in the Chilisai gorge (Osh province). By chloroform extraction we isolated 0.31% of total alkaloids, of which 0.18% formed an ethereal extract. By chromatography on a column of silica gel the ether fraction yielded glaucine, thalicmidine, and oxyacanthine and a new base with the composition $C_{20}H_{19}NO_7$, mp 193-194°C (methanol), which has been called intebrine. The known alkaloids were identified from their physicochemical constants and spectral characteristics and also by comparison with authentic samples.

Intebrine (I) is an optically inactive base of nonphenolic nature crystallizing well from methanol in the form of prisms, sparingly soluble in ethanol, ether, and benzene, and readily soluble in DMSO and pyridine. Intebrine was not methylated by diazomethane or by Craig's method, nor was it acetylated by acetic anhydride in pyridine, although its IR spectrum contained broad absorption bands showing the presence of an active hydrogen atom (ν_{max}^{KBr} 3440, 2430 cm^{-1}). In the UV spectrum of (I) were observed the bands with $\lambda_{max}^{C_2H_5OH}$ 223, 250, and 304 nm ($\log \varepsilon$ 4.98, 4.62, and 4.45, respectively), that are characteristic for isoquinolines [4]. A comparative study of the spectral characteristics and chemical properties of (I) permitted it to be assigned to the 1-benzyl- [5] or N-benzylisoquinoline [6] alkaloids containing a methylenedioxy group in ring A and also dimethoxy and carboxy groups in ring C.

However, its spectral characteristics (see below) and chemical properties did not permit the structure of intebrine to be established unambiguously. In order to determine its structure reliably, we made an x-ray structural investigation. The spatial structure found by the x-ray structural method showed that intebrine belongs to a new type of isoquinoline alkaloids, and its molecule is based on a N-benzylisoquinoline skeleton.



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The structure of the molecule of (I) in a projection on the mean square plane of the tricyclic nucleus is shown in Fig. 1, which also gives the bond lengths; valence angles are listed in Table 1. In the molecule of (I) the benzene rings A and C are planar with an accuracy of ±0.02 Å, while heterocycle B has a conformation close to the sofa type: the C4, C5a, C8a, C1, and N2 atoms are in one plane (±0.07 Å) and the C3 atom deviates from this

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TABLE 1. Valence Angles (ω, degrees) in the (I) Molecule

Angle	ω	Angle	ω	Angle	ω
N2—C3—C4	110,9	C9—C8—C8a	118,4	O7—C12—C11	116,2
C3—C4—C5	111,4	N2—C1—C8a	119,9	O7—C12—C13	125,8
C4—C5a—C5	120,7	O3—C1—C8a	118,0	C14—C13—C12	120,9
C4—C5a—C8a	120,1	O3—C1—N2	122,1	C15—C14—C13	120,4
C5—C5a—C8a	119,1	N2—C9—C10	112,5	C14—C15—C10	120,3
C5a—C5—C6	117,2	C1—N2—C3	122,0	C16—C15—C10	125,0
C5—C6—C7	122,0	C9—N2—C3	115,0	C16—C15—C14	114,7
O1—C6—C6	126,9	C1—N2—C9	119,5	O4—C16—C15	118,0
O1—C6—C7	111,0	C9—C10—C11	115,2	O5—C16—C15	122,6
C6—C7—C8	122,9	C15—C10—C9	125,6	O5—C16—O4	119,3
O2—C7—C6	109,8	C15—C10—C11	118,7	O2—C17—O1	106,8
O2—C7—C8	127,3	C12—C11—C10	121,8	C17—O1—C6	105,9
C6—C8—C8a	115,7	O6—C11—C10	118,8	C17—O2—C7	105,7
C8—C8a—C5a	123,1	O6—C11—C12	119,3	C18—O6—C11	116,0
C9—C8a—C5a	118,5	C13—C12—C11	117,9	C19—O7—C12	117,5

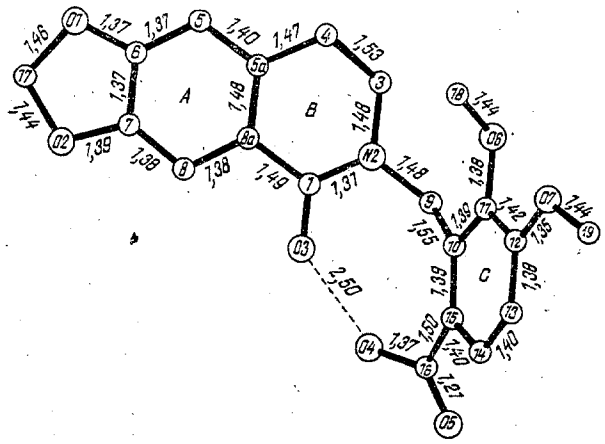


Fig. 1

plane by 0.53 Å. The five-membered methylenedioxy ring has the form of a greatly flattened envelope (±0.01 Å) with a deviation of the C17 atom from the plane of the other four by 0.13 Å.

The mutual positions of the tricyclic system and ring C are characterized by the torsional angles C1N2C9C10 (−70°) and N2C9C10C11 (−71°). The carboxy group (the C15, C16, O4, and O5 atoms) is somewhat (35°) turned relative to the plane of ring C. Such a conformation of the (I) molecule is stabilized by a strong intramolecular H-bond between the amide carbonyl and the OH of the carboxy group: O3...O4 2.50 Å, H...O3 1.51 Å, and the angle O4−H...O3 168°. The observed slight shortening of the valence bonds C1—N2 (1.31 Å) and O4−C16 (1.33 Å) and the lengthening of the C1=O3 bond (1.27 Å) in comparison with the standard values [7] likewise show a powerful interaction of the carboxy group with the amide carbonyl. Here the errors in the determination of bond lengths were not more than 0.013 Å, while for the valence angles they did not exceed 0.9°.

Analysis of the crystal structure of (I) showed the absence of shortened intermolecular contacts and, consequently, of the corresponding H-bonds (see Fig. 1).

The intermolecular H-bond shown well explains the negative result of the reaction of (I) with diazomethane and the position of the maximum of the amide carbonyl band observed in the IR spectrum (1580 cm^{-1}), which is characteristic for chelate bonds, [8].

The PMR spectrum of intebrine showed two-proton triplets at 2.70 and 3.24 ppm from the methylene protons at C3 and C4, respectively; three-proton singlets at 3.68 and 3.85 ppm from two methoxy groups; and two-proton singlets at 4.97 ppm from the methylene protons in the benzyl moiety and at 6.00 ppm from the methylenedioxy group. Aromatic protons were shown at 6.72 and 7.27 ppm in the form of one-proton singlets from C5−H and C8−H, and also in the form of two one-proton doublets at 7.05 and 7.65 ppm from C12−H and C13−H, respectively. It can be seen from the PMR spectrum of (I) that the signals of the aromatic proton at C8 and of the methylene protons of the benzyl moiety were shifted downfield under the influence of the amide carbonyl. A similar shift was observed for C13−H under the influence of the carboxy group.

TABLE 2. Coordinates (×10) of the Nonhydrogen Atoms in the Structure of Intebrine

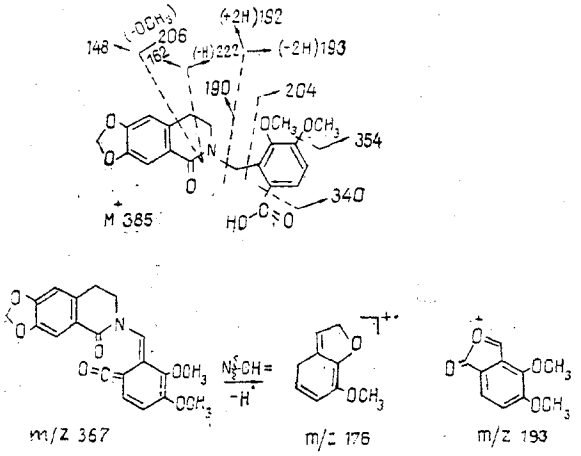
Atom	x	y	z	Atom	x	y	z
C1	2327(12)	9162(4)	2565(10)	C13	6301(13)	11720(5)	2521(11)
N2	2147(10)	9513(4)	3392(9)	C14	4700(13)	11668(5)	1904(11)
C3	1752(15)	9132(5)	4328(0)	C15	3197(13)	11206(5)	2135(10)
C4	2853(15)	8416(5)	4384(11)	C16	1530(15)	11235(6)	1441(11)
C5a	2647(11)	7996(5)	3476(10)	C17	2346(17)	6050(6)	1406(11)
C5	2737(12)	7233(5)	3490(12)	C18	6347(15)	9952(6)	4594(11)
C6	2591(12)	6880(4)	2608(11)	C19	9169(18)	11956(6)	3952(12)
C7	2477(13)	7250(5)	1737(11)	O1	2656(10)	6153(3)	2455(9)
C8	2389(13)	7995(5)	1685(10)	O2	2415(10)	6761(4)	0963(9)
C8a	2488(12)	8357(4)	2568(11)	O3	2322(9)	9485(3)	1746(9)
C9	1624(12)	10295(4)	3379(11)	O4	0582(10)	10620(4)	1259(9)
C10	3271(11)	10781(5)	2986(10)	O5	1075(11)	11782(4)	1014(9)
C11	4838(13)	10857(5)	3606(9)	O6	4844(8)	10485(3)	4477(9)
C12	6382(13)	11334(4)	3387(11)	O7	7774(8)	11386(4)	4068(9)

The mass-spectrometric properties of intebrine are determined by the nature and relative positions of the substituents in the benzyl moiety of the molecule and by the intramolecular H-bond. In analyzing the mass spectrum of 7-hydroxy-6-methoxy-N-(4'-methoxybenzyl)-3,4-dihydroisoquinoline (sendaverine), Kametani and Ohkubo [6] established that the main direction of its fragmentation under electron impact was the cleavage of the N–benzyl bond with the formation of the 100% peak of a methoxytropylium ion with m/z 121 and an isoquinolinium cation with m/z 178. The breakdown of the latter with the ejection of a CH_2N particle leads to an ion with m/z 150. The splitting out of a methoxyphenyl radical, leading to an ion with m/z 192, is of secondary importance.

The cleavage of the N–CH_2(Ar) bond in the M^+ ion of intebrine is also important, but here, together with an ion having m/z 190 (simple cleavage of the bond, Scheme 1), an ion having m/z 192, with the transfer of two hydrogen atoms due, primarily, to the above-mentioned small distance between the –COOH...O=C groups, is formed. In full agreement with this, a nitrogen-free fragment having m/z 193, with the migration of 2H in the opposite direction, also arises.

A large contribution to the total ion current is made by the fragmentation of the substituents in the phenolic nucleus. In contrast to sendaverine, a methoxy radical is split out intensively from the M^+ ion of intebrine (ion with m/z 354), which is characteristic of all the isomers of anisic acids. In its turn, the splitting out of COOH leads to an ion with m/z 340, which is converted into an ion with m/z 310 by the loss of a molecule of formaldehyde.

In all cases, we established the sequence of the acts of fragmentation of intebrine with the aid of the methods of metastable defocussing (MD) and linked scanning, B/E = const, while the origin of the ions was checked by measuring their elementary compositions. It was found, in particular, that the ions with m/z 176, the peaks of which were the strongest



Scheme 1. Scheme of the fragmentation of intebrine under EI (in the m/z 176 ion, =C=O has been omitted).

in the spectrum of (I), are not formed directly from M^+. The maternal ion for the nitrogen-free component $C_{10}H_8O_3$ is the $(M - H_2O)^+$ ion (Scheme 1). As the MD spectrum of the ions with m/z 176 showed, their precursor is likewise a methyleneisoquinoline fragment with m/z 204. Two components may arise from this ion by the elimination of CO or CH_2N: $C_{10}H_{10}NO_2$ and $C_{10}H_8O_3$.

An ion with m/z 193 is formed not only from M^+ but also from the nitrogen-containing product of a retrodiene reaction of ring B – an ion with m/z 222 – by the elimination of CH_3N. In its turn, the ion with m/z 193, by losing a formaldehyde molecule, is converted into an ion with m/z 163, which arises alternatively from a m/z 354 fragment by the cleavage of the N–CH_2 bond.

EXPERIMENTAL

UV spectra were taken on an Hitachi EPS-3T instrument (in ethanol), IR spectra on a UR-20 instrument (in tablets with KBr), PMR spectra on a Tesla BS-567A instrument (in DMSO-d_6, 0 – TMS), and mass spectra on a MKh-1310 instrument (EI source, 160 μA). For the conditions of measuring the elementary compositions of the ions, and for MD and B/E, see [9]. Chromatography was conducted on KSK silica gel with the following solvent systems: chloroform–methanol (98:2); chloroform–ethanol (9:1); and chloroform–methanol–conc. HCl (50:50:0.1).

Isolation of the Total Alkaloids. The air-dry comminuted leaves of B. integerrima (3 kg) were wetted with an 8% solution of ammonia, and after 2 h the alkaloids were extracted with chloroform (3 extractions). The chloroform extracts were combined and evaporated to a volume of 1 liter, and the alkaloids were extracted with 3% hydrochlorid acid. The acid solution was washed with ether and was then made alkaline with a 25% solution of ammonia and was extracted successively with ether and with chloroform. The solutions obtained were dried over Na_2SO_4, filtered, and evaporated. This gave 5.4 g of ether fraction and 3.9 g of chloroform fraction. The total yield of alkaloids was 0.31% on the weight of the dry plant.

Separation of the Total Ether Fraction. The total ether fraction of alkaloids (5.4 g) was chromatographed on a column of silica gel (1:30). The alkaloids were eluted with chloroform and with mixtures of chloroform and methanol in various ratios. The chloroform eluate yielded 2.4 g of glaucine, and the chloroform–methanol (97:3) eluate 0.77 g of oxyacanthine, while the (95:7 [sic]) eluate gave 0.14 g of intebrine. The latter was recrystallized from methanol, mp 193-194°C. IR spectrum (ν_{max}^{KBr}, cm^{-1}): 3440, 2430, 1715, 1580, 1490, 1400, 1280. Mass spectrum m/z (I, %): 385 (M^+, $C_{20}H_{19}NO_7$; 81), 367(20), 356 ($C_{19}H_{18}NO_6$; 30), 354 ($C_{19}H_{16}NO_6$; 100), 340 ($C_{19}H_{18}NO_5$; 22), 338(19), 326(7), 324(13), 323(6), 412(14), 310 ($C_{18}H_{16}NO_4$; 50), 296(7), 282(7), 222 ($C_{11}H_{12}NO_4$; 5), 210 ($C_{10}H_{12}NO_4$; 6), 206 ($C_{11}H_{12}NO_3$;10), 204 ($C_{11}H_{10}NO_3$; 19), 193 ($C_{10}H_9O_4$; 67), 192 ($C_{10}H_{10}NO$; 50), 190 ($C_{10}H_8NO_3$; 72), 176 ($C_{10}H_8O_3$; 64), 176 ($C_{10}H_{10}NO_2$; 32), 175 (38), 163 ($C_9H_7O_3$; 41), 162 ($C_9H_6O_3$; 18), 162 ($C_9H_8NO_2$; 6), 148 (28), 134 (38).

X-Ray Structural Investigation. Colorless crystals of (I) of tabular form were grown from solution in methanol. The space group, the parameters of the unit cell, and the intensities of the reflections of the crystal were measured on a Syntex $P2_1$ automatic four-circle diffractometer at room temperature, using CuK_α radiation: a = 6.948(2), b = 18.400(7), c = 13.645(5) Å, d_{calc} = 1.467 g/cm³, space group $Pna2_1$, Z = 4.

The intensities of 1246 reflections with $\theta < 58°$ were measured on this diffractometer ($\theta/2\theta$ scanning). In the preliminary treatment we excluded weak reflections with $I < 2\sigma(I)$. We used 972 reflections with $|F| > 4\sigma(|F|)$ in the calculations. The structure was determined by the direct method using the SHELXS-86 program [10] and was refined in the full-matrix isotropic-anisotropic approximation by the SHELX-76 program [11] (both programs in the PC MSDOS version). The positions of the H atoms were calculated geometrically, except that in the hydroxy group was found from a difference electron density synthesis. The final value of the residual index R was 0.048 (R_w = 0.048). The coordinates of the nonhdrogen atoms are given in Table 2.

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Berberis ALKALOIDS.

XXII. INTEBRININE AND INTEBRIMINE – NEW ALKALOIDS FROM Berberis integerrima

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The known alkaloids reticuline, isoboldine, isocorydine, glaucine, armepavine, oxyacanthine, and heliamine and the new alkaloids intebrinine and intebrimine have been isolated from the total alkaloids of the leaves of Berberis integerrima, and structures have been proposed for the latter two on the basis of spectral characteristics and independent synthesis.

The alkaloid composition of the leaves of Berberis integerrima Bge gathered in the environs of Sarikurgana (Fergana province) has been investigated. The new N-benzylisoquinoline alkaloid intebrine has been isolated from the leaves of this plant previously [1]. Chloroform extraction of the leaves yielded 0.28% of total alkaloids (0.19% of ether fraction and 0.09% of chloroform fraction). By chromatography on a column of silica gel we isolated reticuline, isoboldine, and isocorydine from the total chloroform fraction, and glaucine, armepavine, oxyacanthine, and heliamine and the two new alkaloids intebrinine (IV) and intebrimine (V) from the total ether fraction.

All the known alkaloids that were isolated were identified on the basis of spectral characteristics and direct comparison with authentic samples. This is the first time that armepavine [2] and heliamine [3] have been obtained from a plant of the genus Berberis.

Intebrinine (IV) was isolated in the form of an oil but its hydrochloride crystallized well from methanol. The IR spectrum of the base lacked an absorption band of active hydrogen. Its UV spectrum was characteristic for the tetrahydroisoquinoline alkaloids [4]. The mass fragmentation of the hydrochloride of (I) under EI resembled that of the N-benzylisoquinoline alkaloids [5]. The presence of the maximum ion with m/z 192 and of intense ions with m/z 164 and 135 formed in cleavages a and b showed that in ring A of interbrinine there were two methoxyls and in ring D a methylenedioxy group.

The fact that (IV) was a N-benzylisoquinoline was confirmed by its PMR spectrum, which contained two two-proton singlets, at 3.60 and 3.51 ppm for the methylene protons at C-1 and C-α, respectively [5]. The nature of the signals of the five aromatic protons in the PMR spectrum of intebrinine gave grounds for stating that the methoxy groups in ring A occupied positions 6 and 7, and the methylenedioxy group in ring C positions 3' and 4'. The assignment of the chemical shifts of the protons in the PMR spectrum is given in Table 1.

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