Aminoalcohol from 6-Acetylumbrofine. A mixture of 0.04 g of 6-acetylumbrofine and 10 ml of 2% aqueous methanolic KOH was heated in the water bath for 2.5 h. The completion of the reaction was monitored by TLC. After the solvent had been distilled off, the residue was dissolved in water and extracted with ether (3 × 40 ml). This gave an aminoalcohol (0.025 g), mp 110-112°C, which was identified as umbrofine by a direct mixed melting point with an authentic sample, by TLC, and by a comparison of mass, IR, and PMR spectra.

IR (KBr): 3548, 3400-3200, 1471, 1392, 1368, 1326, 1302, 1250, 1205, 1170, 1118, 1100, 1068, 1028, 998, 995, 967, 932, 886, 863, 752 cm^{-1}.

M^+ 423(11.2), 408(9.7), 406(9.4), 405(5.9), 392(100), 376(11.0), 374(5.3).

PMR, ppm: $N-CH_2-CH_3-$ 1.06 (3H, t), J = 7.5 Hz; $3OCH_3$ groups at 3.28, 3.37, and 3.42 (3H each, s), β-H-C-14 at 3.65 (t, J = 4.5 Hz), α-H-C-6 at 4.23 (br.s).

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Berberis ALKALOIDS.

XXIII. STRUCTURE OF TURCBERINE

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Berberine, magnoflorine, palmatine, columbamine, jatrorhizine, epiberberine, berbamine, O-methylisothalicberine, armepavine, corypalline, glaucine, and corydine and the new alkaloid turcberine have been isolated from *Berberis turcomanica* Kar., and the structure of turcberine has been established. Apart from berberine, this is the first time that any of these alkaloids have been isolated from this species of barberry, while this is the first time that corydine and armepavine have been isolated from the genus *Berberis*.

We have investigated the alkaloid composition of the leaves and young shoots of *Berberis turcomanica* Kar. growing in Turkmenia (Kopet Dagh, environs of Bendeinsk) in the stage of incipient flowering.

The isolation of berberine from the roots of this plant has been reported previously [1].

Extraction of the leaves successively with chloroform and ethanol yielded 0.27% of total alkaloids, of which 0.07% consisted of berberine. By separating the total mixture so obtained on a column of silica gel we isolated glaucine, corydine, armepavine, and corypalline. The main alkaloid in the leaves was glaucine.

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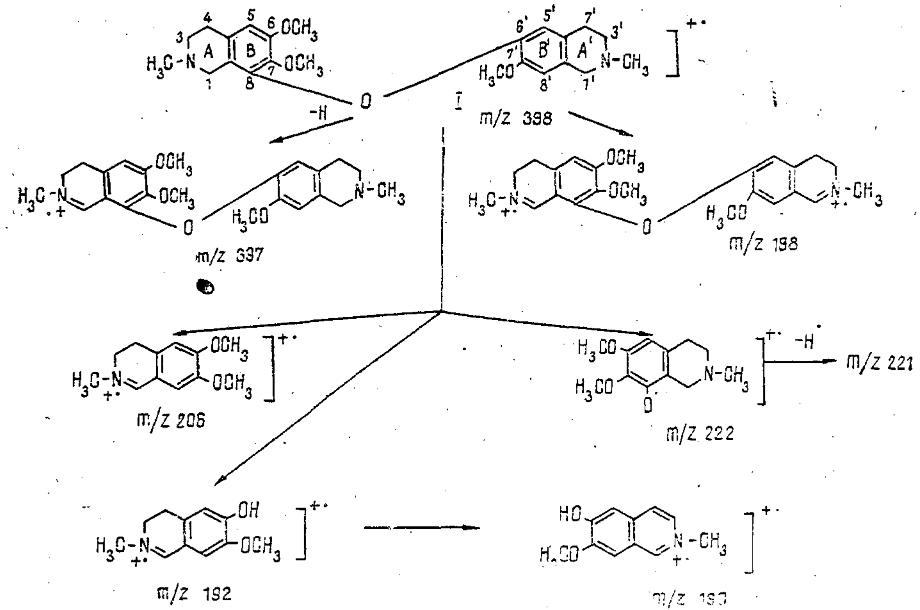
Young shoots were extracted successively with chloroform and methanol. The methanol-extracted material yielded the iodides of berberine, magnoflorine, palmatine, columbamine, jatrorrhizine, and epiberberine. The material obtained by chloroform extraction yielded oxyacanthine, berbamunine, 2'-N-methylberbamine, O-methylthalicberine, and reticuline and the new base turcberine (I).

Turcberine (I), isolated in the form of an oil of nonphenolic nature and optically inactive, had the composition $C_{23}H_{30}N_2O_4$. Its UV spectrum showed absorption in the region of $\lambda_{max}^{C_2H_5OH}$ 283 nm (log ε 3.83). There was no band of active hydrogen in the IR spectrum.

The nature of the fragmentation of (I) under electron impact was similar to that of the bisbenzylisoquinoline alkaloids (scheme) [2]. The presence of ions with m/z 398 (M^+) and 198^{++} and of intense ions with m/z 221, 206, and 192 showed that turcberine consists of two simple tetrahydroisoquinolines and is the first representative of a new type of isoquinoline alkaloid.



The PMR spectrum of turcberine (in $CDCl_3$ at room temperature) consisted of resonance signals readily assignable to characteristic groups according to the type of substitution. Three aromatic protons resonated in the form of individual resolved singlets at 6.20, 6.50, and 6.54 ppm. Three three-proton singlets at 3.85, 3.78, and 3.61 ppm related to methoxy groups. Methylene protons in the C-1 and C-1' positions experienced increments of the downfield shifts both from the aromatic rings and from the nitrogen atoms in α-positions to them. Consequently, among the signals of methylene protons, their signals were shifted into the weakest field and formed two slightly broadened singlets at 3.36 and 3.44 ppm.

The singlet character of these signals showed the equivalence of the protons in each geminal pair and, consequently, the rapid conversion of the saturated rings of the molecule. The other eight methylene protons of the molecule (in the C-3, C-3', C-4, and C-4' positions) formed two signals: an uneven two-proton triplet (J = 5.5 Hz) at 2.83 ppm and a complex multiplet formed by the overlapping of a triplet conjugate to a triplet at 2.57 ppm and a four-proton singlet, which had the same chemical shift, at 2.57 ppm. Two three-proton singlets at 2.32 and 2.36 ppm related to two N-methyl groups.

The assignment of the signals to protons within characteristic stereochemical groups was made on the basis of nuclear Overhauser effect (NOE) measurements and a comparative analysis with the PMR spectrum of a model compound, heliamine (II), which we recently isolated from B. integerrima [3].

The NOE results on observation of the signals of aromatic protons and irradiation of the saturated chains of the turcberine (I) molecule are given below (ppm):

Signals irradiated	Signals observed 6.54	 6.50	 6.20
3.85 (3H, s, OCH_3)	16%	–	–
3.78 (3H, s, OCH_3)	–	14%	–
3.44 (2H, s, CH_2)	12%	–	–
2.83 (2H, t, CH_2)	–	12%	–
2.57 (4H, s, $2CH_2$)	–	–	17%

The ortho-positions of the aromatic protons (6.50 and 6.54 ppm) with respect to the respective methoxyls (3.75 and 3.88 ppm) are obvious.

It must be mentioned that the aromatic proton resonating at 6.54 ppm gave a response simultaneously both to the signal of a methoxyl at 3.85 ppm and to that of a methylene group at 3.44 ppm, which showed the presence of these protons in the same half of the (I) molecule, i.e., in the C-8, C-7, and C-1 or the C-8', C7', and C-1' positions, respectively.

The methoxy group resonating at 3.61 ppm exhibited no NOE with any of the signals of the aromatic and methylene protons. It was therefore spatially isolated from methylene and aromatic protons. A methylene proton resonating at 3.36 ppm likewise exhibited no NOEs, which gave grounds for assuming that one of the C-8 and C-8' positions was substituted by an ethereal oxygen.

The signals of aromatic protons at 6.50 and 6.20 ppm responded to the irradiation with a ratio-frequency field of the triplet at 2.83 ppm and of the complex signal of methylene protons at 2.57 ppm, respectively. Consequently, these aromatic protons had to be assigned to the C-5 and C-5' positions.

In order to interpret the signals of the methylene protons in the C-3, C-3', C-4, and C-4' positions we made a comparative analysis with the spectrum of heliamine (6,7-dimethoxytetrahydroisoquinoline).

The PMR spectrum of heliamine taken in $CDCl_3$ showed signals at (ppm): 6.55 (1H, s), 6.48 (1H, s), 3.78 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.61 (2H, s, CH_2), 3.20 (1H, s, NH) and 2.77 (4H, s, $2CH_2$). Characteristic for the PMR spectrum of (II) is the fact that all four methylene protons in the C3 and C4 positions had the same chemical shift and formed a narrow singlet signal at 2.77 ppm. The presence of a broadened four-proton signal of methylene protons in the PMR spectrum of turcberine agreed well with the random degeneracy of the methylene protons in the PMR spectrum of heliamine, and it was possible to ascribe it to the dioxygen-substituted half of (I) – i.e., to the C-3' and C-4' positions.

In this case, the uneven triplet at 2.83 ppm and the triplet conjugate to it and overlapping with the four-proton singlet at 2.57 ppm had to be assigned to C-3 and C-4 positions of the trioxygen-substituted half of turcberine (I). The elimination of degeneracy was apparently due to the additional oxygen-substituion of the C-8 position and the redistribution of electron density in the aromatic ring (ring B) connected with this.

From the facts given above it was possible to make a definitive assignment of the signals in the PMR spectrum of turcberine (Table 1).

The assignment of the $N-CH_3$ signals in the PMR spectrum of turcberine was made from the weak NOE (~3-4%) on the irradiation of the methylene protons (3.44 and 3.36 ppm) and observation of the N-methyl signals.

On the basis of the facts given, turcberine has the structure (I). The alkaloid berbidine, consisting of a dimer of simple isoquinoline and isoquinolone has previously been isolated from the Pakistan barberry <u>B. brandisiana</u> [4]. The authors concerned put forward the hypothesis that berbidine was formed from chenabinol by in vintro oxidation.

In its chemical structure, turcberine is the first of a new type of dimeric isoquinolines consisting of two simple tetrahydroisoquinolines. The latter may be formed in the plant both from alkaloids of the type of isothalicberine and by the condensation of two simple isoquinolinealkaloids, as is shown by the presence in this plant of alkaloids of both these groups: O-methylthalicberine and corypalline.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. Specific optical rotations were determined on a Jasco J-20 spectropolarimeter. UV spectra were taken on a Hitachi EPS-3T

TABLE 1. Assignment of the Signals of the Protons in the PMR Spectra of Turcberine and Heliamine

Position in the molecule	Chemical shift, ppm	Position in the molecule	Chemical shift, ppm	Position in the molecule	Chemical shift, ppm
		Turcberine		Heliamine	
C_1-H_2	3,36 (2H,s)	$C_1'-H^2$	3,44 (2H,s)	C_1-H_2	3,61 (2H,s)
$2\text{-}N-CH_3$	2,32 (3H,s)	$2'\text{-}N-CH_3$	2,36 (3H,s)	2-NH	3,20 (1H,s)
C_3-H_2	2,57(2H,t, J=5,5 Hz)	$C_3'-H_2$	2,57 (4H,s)	C_3-H_2	2,77 (4H,s)
C_4-H_2	2,83(2H,t, J=5,5 Hz)	$C_4'-H_2$		C_4-H_2	
C_5-H	6,50(1H,s)	$C_5'-H$	6,20 (1H,s)	C_5-H	6,55 (1H,s)
C_6-OCH_3	3,78(3H,s)	$C_7'-OCH_3$	3,85 (3H,s)		
C_7-OCH_3	3,61(3H,s)	$C_8'-H$	6,54 (1H,s)	C_6-OCH_3	3,78 (3H,s)
				C_7-OCH_3	3,76 (3H,s)
				C_8-H	6,48 (1H,s)

instrument. The IR spectra of the compounds under investigation were recorded on a UR-20 spectrometer in the 3700-750 cm^{-1} region using tablets molded with potassium bromide. The mass spectra of the alkaloids and their derivatives were obtained on a MKh-1310 double-focusing mass spectrometer with a system for the direct introduction of the sample into the ion source, at an ionizing energy of 60-70 eV and a temperature of 160-170°C. NMR spectra were taken on a Tesla BS A/100 MHz instrument.

The concentrations of the substances ranged between 3 and 4%. HMDS was used as internal standard. NOEs were recorded in the regime of constant irradiation and the integral change in the intensity of the signals. In the measurement of NOEs on the poorly resolved signals of N-methyl protons, the evaluations of the NOEs were made from the amplitude intensities of the signals.

The purity of the alkaloids was checked with the aid of TLC on plates coated with type KSK silica gel and with alumina (Brockmann activity grade II). Revealing agents: the Dragendorff reagent and iodine vapor. Solvent systems: 1) chloroform–methanol (95:5 and 9:1); 2) chloroform–methanol–conc. HCl (50:50:0.1); 3) benzene–ethanol (9:1). Column chromatography was conducted on the sorbents silica gel and alumina with particle sizes of 125-160 μm.

Isolation and Separation of the Alkaloids from B. turcomanica. a) From the Leaves. B. turcomanica leaves (800 g) were wetted with 8% ammonia and were extracted successively with chloroform and ethanol. The chloroform extract yielded 1.59 g, and the ethanol extract, by precipitation with potassium iodide, 0.57 g of berberine iodide (total yield 0.27%).

When the chloroform alkaloids were separated, armepavine (0.3 g), corypalline (0.2 g), glaucine (0.8 g), and corydine (0.1 g) were isolated.

b) From Young Shoots. The comminuted young shoots (1400 g) were extracted successively with chloroform and methanol by the procedure described in [5]. The chloroform extract then yielded 5.2 g of an ether fraction (A) and 2.8 g of a chloroform fraction (B). The methanolic extract gave 4.06 g of berberine iodides and 2.40 g of magnoflorine, and also 3.74 g of a mixture (C) of quaternary iodides. The ether fraction (A) (5.2 g) was separated into phenolic (D) (1.4 g) and nonphenolic (E) (3.8 g) parts.

Separation of the Nonphenolic Part (E) of the Ether Fraction (A). The material of part (E) was separated on a column of Al_2O_3 (Brockmann activity grade II), with elution by chloroform and by mixtures of chloroform and methanol in various ratios. The chloroform eluates yielded a crystalline base with mp 205-207°C, $[\alpha]_D$ −28° (c 0.2, $CHCl_3$), M^+ 622, which was identified as O-methylthalicberine (0.2 g). From the fractions eluted by chloroform–methanol (98.2) we isolated oxyacanthine (0.5 g); (97:7) berbamunine (1.6 g); (96:4) reticuline (0.8 g); and (95:5) turcberine (0.1 g) in the form of an oil.

IR (ν_{max}^{KBr}, cm^{-1}): 2850, 1610, 1140, 800, 810.

UV ($\lambda_{max}^{C_2H_5OH}$, nm): 283 (log ε 3.8).

Mass spectrum, m/z (%): 398 (M^+, 23), 397(57), 383(6), 368(29), 220(13.5), 221(100%), 222(44.5), 206(96.7), 190(19), 192(13), 198(13), 174(13), 175(15), 176(28).

Separation of the Chloroform Fraction (B). Part of the chloroform fraction (1.8 g) was separated on a column of alumina, elution being conducted in a similar way to that for part (E). Chloroform–methanol (98:2) led to the isolation of oxyacanthine (0.8 g), and (97:3) to 2'-N-methylberbamine (0.2 g) and berbamunine (0.7 g).

Separation of the Mixture of Quaternary Iodides (C). Part of mixture (C) (2.2 g) was separated on a column of silica gel, elution being conducted as in the preceding cases. The chloroform–methanol (98:2) fractions yielded palmatine (0.6 g) and epiberberine (0.2 g) and the (96:4) fraction jatrorrhizine (0.3 g) and columbamine (0.4 g).

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