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Berberis ALKALOIDS

XXXII. BERBERAL — A NEW ALKALOID FROM *Berberis heterobotrys*

A. Karimov, M. F. Faskhutdinov, N. D. Abdullaev,
M. G. Levkovich, E. G. Mil'grom, Ya V. Rashkes,
and R. Shakirov

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Berberine, palmatine, jatrorrhizine, oxyacanthine, berbamine, reticuline, obaberine, isocorydine, thalicmidine, and the new base berberal (I) have been isolated from leaves and young shoots of Berberis heterobotrys Wolf. The structure of berberal has been established by a study of its ^1H and ^{13}C NMR spectra and measurement of intramolecular NOEs, and also by mass-spectrometric analysis.

Berberis heterobotrys Wolf. (fam. *Berberidaceae*), a prickly shrub about 3-3.5 m high, grows in Central Asia and is widely distributed on the territory of Tadzhikistan [1, 2]. In folk medicine the roots of this plant are used for the treatment of rheumatism, skin diseases, malaria, dysentery, and infertility, and the leaves and fruit as an antiscorbutic.

Berberine has previously been isolated from the roots and the bark of the stems of *B. heterobotrys*, and the presence of tertiary and quaternary bases has been shown [3, 4], while, in addition to berberine, palmatine has been isolated from the fruit [5]. Continuing investigations of the alkaoids of plants of the *Berberis* genus, we have now studied the alkaloid composition of young shoots and leaves gathered in the incipient fruit-bearing phase on the Zarafshan range, in the valley of the river Fondar'ya (Tadzhikistan).

By extracting young shoots with ethanol we isolated 0.49% of alkaloids, of which 0.09% consisted of berberine. By chromatography on a column of silica gel the total tertiary alkaloids yielded oxyacanthine, berbamine, reticuline, and obaberine, while the total quaternary alkaloids yielded palmatine, jatrorrhizine and berberine in the form of chlorides. Chloroform extraction of the leaves gave 0.12% of alkaloids. The chromatography of this material on a column of silica gel led to the isolation of isocorydine, thalicmidine, and a new crystalline base (I). The presence of berberine and palmatine in the leaves was detected chromatographically.

The IR spectrum of (I) contained absorption bands of amide and lactone carbonyls (1660 and 1770 cm^{-1}, respectively). In the UV spectrum there were the absorption maxima that are characteristic for isoquinolines [6].

The mass spectrum of the base, with the M^+ ion having m/z 383, composition $C_{20}H_{17}O_7N$, resembled that of intebrine [7] but differed from it by two hydrogen atoms. The ion with m/z 383 broke down into two mutually complementing fragments with m/z 190 ($C_{10}H_8O_3N$) and 193 ($C_{10}H_9O_4$). Thus, the 2H deficiency relates to the nitrogen-free part of the molecule, and, in combination with the position of the maximum at 1770 cm^{-1}, could show the presence of a γ-lactone ring formed by the dehydrogenation of a COOH group and one H-9 of intebrine.

The structure of (I) was studied independently from the ^1H and ^{13}C NMR spectra and also from several variants of the measurement of nuclear Overhauser effects.

The PMR spectrum of the base (Table 1) was represented in the aromatic region by three one-proton singlets at 6.60, 7.59, and, broadened, at 7.97 ppm, and two one-proton doublets at 7.14 and 7.65 ppm with a spin-spin coupling constant (SSCC) J = 8.0 Hz corresponding to an aromatic AB system. A two-proton singlet appeared in the same region, at 6.01 ppm, corresponding to a methylenedioxy group. In the aliphatic region there were two three-proton singlets from methoxy groups at 3.87 and 3.97 ppm, two one-proton sigals in the form of a doublet of triplets with the geminal SSCC ^2J = 14.7 Hz and two vicinal SSCCs, ^3J = 7.4 Hz, at 2.99 and 3.20 ppm, and one two-proton triplets at 2.81 ppm with the SSCC ^3J = 7.4 Hz.

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TABLE 1. Chemical Shifts and SSCCs of the Protons and Carbon Atoms in the ^1H and ^{13}C NMR Spectra of Berberal in $CDCl_3$

Protons	Chemical shifts (multiplicity, J. Hz), δ, ppm	Carbon atoms	Chemical shifts (multiplicity, J. Hz), δ, ppm
H-3	2.99; 3,20 (2H, d.t , 14.7 Hz; 7.4 Hz)	C-1′	164.5a
H-4	2.81 (2H, t, 7.4 Hz)	C-10	168.4^a
H-5	6.60 (1H, s)	C-6	151.3^b
H-8	7.59 (1H, s)	C-7	147.1^b
H-9	7.97 (1H, s)	C-6′	144.0^b
H-4′	7.14 (1H, d, 8.0 Hz)	C-5′	157.3^b
H-3′	7.65 (1H, d, 8.0 Hz)	C-4a	134.5^c
6′-OCH_3	3.87 (3H6 s)	C-8a	122.4^c
5′-OCH_3	3.97 (3H, s)	C-1′	135.8^c
(6,7)-OCH_2O-	6.01 (2H, c)	C-2′	120.7^c
		C-5	108.6^d (d, 168.75 Hz)
		C-8	107.0^d (d, 163.36 Hz)
		C-4′	115.0^d (d, 161.57 Hz)
		C-3′	121.8^d (d, 172.34 Hz)
		C-9	81.8 (d, 165.15 Hz)
		OCH_2O (C_6—C_7)	101.7 (t, 174.13 Hz)
		6′-OCH_3	56.5 (q, 144.81 Hz)
		5′-OCH_3	60.8 (q, 146.01 Hz)
		C-3	40.0 (t, 138.23 Hz)
		C-4	27.9 (t, 154.25 Hz)

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*a, b, c, d — possible alternative variants of the assignment of the signals.

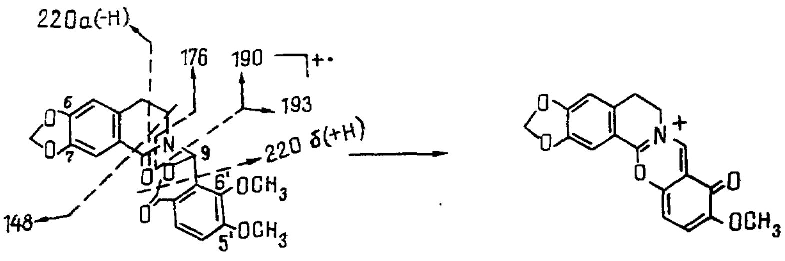
To determine the position of the methylenedioxy and methoxy groups we investigated NOEs both in the NOESY two-dimensional variant and in the usual regime for the quantitative determination of the effect.

When the triplet at 2.81 ppm was irradiated with a radiofrequency field a NOE was observed on the aromatic singlet at 6.60 ppm (16%), which showed that this proton was present in the peri- position to the 2H-4 protons of ring *B*. This meant that the singlet at 6.60 ppm had to be assigned to the H-5 proton, and the triplet at 2.81 ppm to the 2H-4 methylene protons. The singlet form both of the signal of the H-5 proton and of that at 7.59 ppm showed their para- positions. Thus, the second aromatic singlet related to a proton at C-8, and the C-6 and C-7 positions must have been substituted by the methylenedioxy group the methylene protons of which resonated at 6.01 ppm. The two complex multiplets at 2.99 and 3.20 ppm, obviously linked by vicinal SSCCs with the two H-4 protons, related to the methylene protons in the H-3 position. The different chemical shifts of the H-3 protons showed that the inversion of ring *B* was hindered.

Suppression of the singlet of a methoxy group at 3.87 ppm gave a NOE on the broadened singlet at 7.97 ppm (7%). This meant that the broadened singlet at 7.97 ppm corresponded to the H-9 proton in the phthalide group, while the methoxy group giving the signal at 3.87 ppm had to be present in the C-6′ position. Irradiation of the signal of methoxy protons at 3.97 ppm showed a NOE on the doublet at 7.14 ppm (16%). On the basis that the aromatic AB system responded by a NOE to only one methoxyl, it was possible to conclude that the second methoxyl, giving a signal at 3.97 ppm, was present in the C-5′ position, while the aromatic protons of the AB system were located in the H-4′ (7.14 ppm) and H-3′ (7.65 ppm) positions.

The ^{13}C NMR spectrum contained 20 signals. From their chemical shifts and in accordance with the nature of the substitution it was possible to group them in the way shown in Table 1. The following were observed in the aromatic part of the spectrum: two carbonyl signals, four signals of quaternary carbons with oxygen substitution, four signals of quaternary carbons, and four signals of tertiary carbons. The signal of the methylenedioxy carbon appeared in the same region. The signals of the C-9 carbon of the phthalide group and of two methoxy carbons and two signals of the methylene carbons of ring *B* appeared in the aliphatic part of the spectrum.

According to the results given above, base (I) had structure (I), i.e., it was berberal. Berberal (I) has been obtained previously in the determination of the chemical structure of berberine by Perkin [9], but this is the first time that it has been detected in plants. By its structure, (I) belongs to a new type of isoquinoline alkaloids — N-phthalidoisoquinolines.



I. $M^{+\cdot}$ 383

m/z 324

The mass spectrum of berberal (I) possessed a number of features distinguishing it from that of intebrine and agreeing with structure (I). The conversion of the carboxy group into a lactone ring led to an increase in the stability of $M^{+\cdot}$. The cleavage of the N–C-9 bond and the formation of fragments with m/z 190 and 193 took place without the migration of two hydrogen atoms as in the case of intebrine [7]. In the high-mass-number region of the spectrum the peaks of ions with m/z 338 $(M - COOH)^+$ and 324 $(M - CO_2 - CH_3)^+$ stood out. The most probable structure of the latter is illustrated in the scheme. However, there are alternative variants of ions with this composition since the MD spectrum of the m/z 324 ion included metastable transitions showing the successive elimination of the particles CHO and CH_2O from $M^{+\cdot}$. In the central part of the spectrum, the peak of an ion with m/z 220, composition $C_{11}H_{10}O_4N$ stood out. This ion was could have been an analogue of the ion with m/z 222 in the spectrum of intebrine, but the possibility is not excluded that it was formed by cleavage of the bonds of the lactone ring (scheme) and thereby confirmed structure (I).

In order to confirm the existence of two variants of the formation of ions with m/z 220 (a and b), we obtained the MD spectra of the ions with m/z 190 and 193, and in both cases recorded the weak peak of a transition from the parental ion with m/z 220. In their turn, the m/z 220 ions had as their precursors the molecular ion and an ion with m/z 354, which likewise did not contradict the existence of two variants of this daughter ion. Here, the origin of the main component of the ions with m/z 176 ($C_{10}H_8O_3$) was different from that for intebrine. It was formed from the isoquinoline part of the molecule by the cleavage of two C–N bonds (scheme) About 15% of the ions with m/z 176 had the composition $C_{10}H_{10}O_2N$ and were formed from the ion with m/z 220 (a) by the ejection of a CO_2 molecule. One of the most considerable fragmentary ions of berberal, with m/z 165 ($C_9H_9O_3$) was formed from the ion with m/z 193 by the loss of CO. An ion with m/z 148 ($C_9H_8O_2$) arose from the isoquinoline moiety as shown in the scheme.

EXPERIMENTAL

For general observations, see [7, 8].

^1H and ^{13}C NMR spectra were taken on Tesla BS 567 A/100 MHz and Bruker WM 500 instruments. The concentrations of the substances ranged between 3 and 4%, and HMDS was used as internal standard. The mass-spectrometric information was obtained on a MKh-1310 instrument. For the experimental conditions, see [7].

Isolation and Separation of the Alkaloids from *B. heterobotrys*. a). From Young Shoots. The air-dry comminuted young shoots were wetted with 600 ml of 5% acetic acid in ethanol. After 2 h, the plant was covered with 3 liters of ethanol, and the mixture was boiled on the water bath under reflux for 2 h. The extract, after cooling to room temperature, was decanted off, and 2 liters of ethanol was added to the plant. Extraction was carried out as described above. Three extracts were obtained in this way. Then the combined alcoholic extracts were evaporated to a viscous state, 100 ml of 5% hydrochloric acid was added, and the mixture was left overnight at 0-5°C. The 0.68 g of yellow crystalline mass (A) that had deposited was then filtered off. The filtrate was made alkaline with ammonia to pH 9, and the alkaloids were extracted with chloroform. After the solvent had been driven off, 1.80 g of tertiary alkaloids (B) was obtained. Then the alkaloids were extracted from the alkaline mother solution with a 9:1 mixture of chloroform and methanol. This gave 0.80 g of quaternary alkaloids (C). The recrystallization of the yellow precipitate (A) from 60% ethanol yielded 0.42 g of berberine chloride.

The 1.80 g of tertiary alkaloids (B) was chromatographed on a column of silica gel (100 g). The alkaloids were eluted with mixtures of chloroform and methanol in various ratios. At a ratio of 99:1, 0.2 g of obaberine was obtained; at 98:2, 0.5 g of oxyacanthine and 0.1 g of berbamine; and at 96:4, 0.12 g of reticuline.

When the quaternary alkaloids were chromatographed in the same way as the tertiary alkaloids, 0.12 g of berberine, 0.14 g of palmatine, and 0.12 g of jatrorrhizine were isolated.

b) From the Leaves. The air-dry comminuted leaves (870 g) were extracted with methanol (three times). The combined methanolic extracts were evaporated to a viscous residue, a 5% solution of acetic acid was added, the mixture was filtered and made alkaline with conc. NH_4OH, and the alkaloids were extracted successively with ether and chloroform. After the drying and filtration of the extracts obtained and elimination of the solvent, 0.61 g of ether-extracted alkaloids and 0.37 g of chloroform-extracted alkaloids were obtained. The ether-extracted alkaloids were chromatographed on a column of silica gel (12 g), the alkaloids being eluted with chloroform and mixtures of chloroform and ethanol in various ratios. The chloroform eluates yielded 0.02 g of berberal; chloroform–methanol (98:2), 0.1 g of isocorydine; (97:3), 0.07 g of thalicmidine; and (96:4), 0.12 g of reticuline.

When the chloroform-extracted alkaloids were chromatographed analogously, 0.12 of thalicmidine was isolated, and berberine and palmatine were detected chromatographically.

Berberal — mp 151-153°C (ether).

IR, ν_{max}^{KBr}: 2970, 2840, 1770, 1660, 1500, 1350, 1270, 1100, 850 cm^{-1}.

UV ($\lambda_{max}^{C_2H_5OH}$, nm): 226, 255, 305 (lg ε 4.92; 4.60; 4.41).

Mass spectrum, m/z (I, %): 383 (M^+, 100), 365 (7), 354 (5), 352 (12), 338 (31), 324 (31), 220 (28), 208 (5), 193 (45), 190 (58), 176 (75), 165 (72), 148 (43).

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