BERBERIS ALKALOIDS.

XXVI. AN INVESTIGATION OF THE ALKALOIDS OF *Berberis amurensis*

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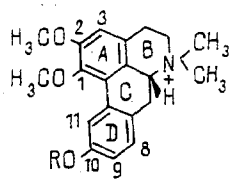
In addition to alkaloids isolated previously, young shoots of Berberis amurensis Rupr. have yielded berberrubine, oxyacanthine, and pseudopalmatine, and the new amorphous base amurenine. It's structure has been established on the basis of spectral characteristics and chemical transformations.

Berberis amurensis Rupr. belongs to the family Berberidaceae and grows in the Far East. The fruit, leaves, and roots of this plant have long been used in folk medicine for the treatment of hypertonia and diseases of the liver and also as a hemostatic agent. In scientific medicine a tincture of the leaves is used in metrorrhagia [1]. Berberine, jatrorrhizine, palmatine, magnoflorine, berbamine, and berbamunine have been isolated from this plant previously [2-6].

In the present paper we give the results of an investigation of the alkaloids from young shoots of *B. amurensis* growing in the environs of the village of Barabash (Maritime Territory, Khasankii region). The total alkaloids (0.34%) were isolated by ethanol extraction. Chromatography of the mixture on a column of silica gel led to the isolation of berberine, berberrubine, pseudopalmatine, and jatrorrhizine and the new base amurenine (I) in the form of chlorides. The tertiary alkaloids oxyacanthine and berbamunine were also isolated. The known alkaloids isolated from this plant were identified by their physicochemical constants and spectral characteristics, and also by comparison with authentic specimens [7]. Among them, this is the first time that berberrubine, oxyacanthine, and pseudopalmatine have been isolated from this plant.

Amurenine (I), an optically active crystalline base possessing phenolic properties, gave a crystalline O-acetyl derivative (II). In the IR spectrum of (I) there were absorption bands at (cm^{-1}) 3200 (active hydrogen), and 1470, 1580, and 1610 (biphenyl system) [6]. The UV spectrum exhibited maxima at (nm) 265, 275, and 305 (log ε 4.23, 4.25, and 4.1), which are characteristic for aporphine alkaloids monosubstituted in ring *D* [8].



I. R=H
II. R=$COCH_3$

On the mass fragmentation of (I) under electron impact, in addition to the formation of ions with *m/z* 326 (M^+), 325 (M — 1), 309 (M — 15), 296, and 298 (M — 58), an ion was produced with *m/z* 58, which is characteristic for quaternary aporphines [9]. Details of the PMR spectra of (I) and (II) taken in CD_3OD and the assignments of the signals of the protons are given in Table 1.

From the values of their chemical shifts, the three-proton singlet in the PMR spectrum of (I) at 3.60 ppm and the one-proton singlet at 6.82 ppm were assigned to C_1—OCH_3 and C_3—H, respectively. The signals of three aromatic protons appeared in the form of an ABC system. The methoxy group giving a signal at 3.82 ppm and the hydroxy group could be present at C-2, C-9, or C-10. To establish the positions of the hydroxy and methoxy groups in rings *A* and *D* we studied the

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TABLE 1. Details of the PMR Spectra of Amurenine (I) and O-Acetylamurenine (II)

Protons	Chemical shift (ppm) and multiplicity (J, Hz)	
	I	II
$N(CH_3)_2$	3,00 (3H, s)	3,02 (3H, s)
	3,36 (3H, s)	3,38 (3H, s)
$C_1 - OCH_3$	3,60 (3H, s)	3,60 (3H, s)
$C_2 - OCH_3$	3,82 (3H, s)	3,83 (3H, s)
$C_3 - H$	6,82 (1H, s)	6,88 (1H, s)
$C_8 - H$	7,11 (1H, dd, 8,5; 1)	7,35 (1H, dd, 8,5; 1)
$C_9 - H$	6,65 (1H, dd, 8,5; 1,5)	6,96 (1H, dd, 8,5; 1,8)
$C_{11} - H$	7,72 (1H, d, 1,5)	7,95 (1H, d, 1,8)
$C_{10} - OCOCH_3$		2,23 (3H, s)

PMR spectra of (I) and (II), and we also measured the nuclear Overhauser effects (NOEs) between the methoxyl, aromatic, and methylene protons in O-acetylamurenine (II).

When the methoxyl giving a signal at 3.82 ppm was irradiated with a radiofrequency field, a NOE (18%) was observed on the broadened singlet at 6.88 ppm, which corresponds to positions of the methoxyl at C-2 and of the aromatic proton at C-3. This was also confirmed by a 76% increase in the intensity of the H-3 signal when the methylene protons with signals at 2.90-3.85 were irradiated because of NOEs and suppression of the small SSCCs from the H-4 protons.

Measurement of a NOE (2%) from the second methoxyl (3.60 ppm) on the aromatic doublet (7.95 ppm; J = 1.8 Hz) showed that the second methoxyl and the doublet aromatic proton were present at C-1 and C-11, respectively. The value of the constant, J = 1.8 Hz, showed the meta position of the closest proton of the aromatic ABC system. This partner could only be the H-9 proton. The signal of this proton was represented by a well resolved doublet of doublets at 6.96 ppm with constants of 8.5 and 1.8 Hz. The third aromatic signal of the ABC system appeared at 7.35 ppm, with constants of 8.5 and 1.0 Hz, which corresponds to the H-8 proton.

The small constant (1.0 Hz) of the H-8 proton arises from interaction with an H-7 proton of the methylene group. This was confirmed by a 62% increase in the intensity of the signal of the H-8 proton on the powerful irradiation of the region of the methylene protons through the suppression of the small constant from the H-7 protons and a NOE. Consequently, the acetyl-substituted position in ring *D* is the C-10 carbon, and on this, therefore, is also located the hydroxy group in amurenine (I).

The signals of the N-methyl groups were located at 3.00 and 3.36 ppm and consisted of slightly broadened singlets.

The downfield shifts of the signals of the aromatic protons in ring *D* on passing from the spectrum of (I) to that of (II) also corresponded to the positions of the substituents given above. For the doublets at 7.72, 7.11, and 6.65 ppm the downfield shifts were 0.23, 0.24, and 0.31 ppm, respectively, while for the singlet at 6.82 ppm the downfield shift was only 0.06 ppm.

It has been shown previously that in the aporphine alkaloids the sign of the specific rotation determines the absolute configuration [8]. Consequently, in amurenine the asymmetric center at C-6a has the R-configuration.

Thus, on the basis of physicochemical and spectral characteristics and chemical transformations we propose structure (I) for amurenine.

EXPERIMENTAL

IR spectra were taken on a UR-20 spectrometer (tablets with KBr), UV spectra on a Hitachi EPS-3T spectrometer (ethanol), mass spectra on a MKh-1310 spectrometer with a system for direct introduction into the ion source, at an ionizing energy of 60-70 eV and a temperature of 160-170°C, and PMR spectra on a BS-567A Tesla spectrometer (Czechoslovakia) in deuteromethanol.

Chemical shifts are given relative to the internal standard HMDS in the δ scale.

For TLS we used type LS 5/40 silica gel (Czechoslovakia), and for column chromatography type KSK silica gel with a particle size of 125-160 μm and the following solvent systems: 1) chloroform—methanol (9:1 and 4:1); 2) chloroform—methanol—conc. HCl (50:50:0.1); and 3) chloroform—ethanol (4:1).

Extraction of Young shoots of *B. amurensis*. The comminuted young shoots (1240 g) were extracted three times with ethanol. The combined alcoholic extracts were evaporated, and the viscous residue was treated with 5% hydrochloric acid. The acid solution was filtered and was washed twice with ether. Then it was made alkaline to pH 9 with 25% ammonia, and the alkaloids were extracted successively with ether, chloroform, and a 4:1 mixture of chloroform and ethanol. After the solvents had been distilled off, 0.68 g of ether fraction, 2.11 g of chloroform fraction, and 1.34 g of quaternary alkaloids were obtained. The total amount of alkaloids made up 0.34% of the weight of the dry plant.

Separation of the Total Alkaloids. The ether fraction (0.68 g) was chromatographed on a column of silica gel (20 g). The alkaloids were eluted with chloroform and with a mixture of chloroform and methanol in various ratios. Elution with chloroform—methanol (97:3) yielded 0.23 g of oxyacanthine, and the 96:4 mixture 0.34 g of berbamunine.

The chloroform fraction (2.15 g) was chromatographed on a column of silica gel in a similar way to the ether fraction. This led to the isolation of 0.72 g of berberine, 0.14 g of pseudopalmatine, 0.24 g of berberrubine, 0.12 g of jatrorrhizine, and 0.07 g of amurenine in the form of chlorides.

The separation of the total quaternary alkaloids on a column of silica gel in a similar way to the chloroform fraction led to the isolation of an additional 0.31 g of berberine, 0.26 g of jatrorrhizine, and 0.12 g of pseudopalmatine in the form of chlorides.

Amurenine Chloride. mp 194-195°C (acetone), $[\alpha]_D$ −164° (*c* 0.03; CH_3OH).

Acetylation of Amurenine Chloride. A mixture of 0.04 g of amurenine chloride, 2 ml of acetic anhydride, and 0.5 ml of pyridine was boiled until the base had dissolved completely and it was then evaporated on the water bath to dryness. The residue was dissolved in 5 ml of acetone with heating. On cooling, warty crystals of O-acetylamurenine chloride (II) deposited. mp 186-187°C. Mass spectrum, *m/z*: 368 (M^+), 367, 359, 352, 325, 58 (100%). IR spectrum: 1760 cm^{-1}.

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