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ALKALOIDS OF Papaver fugax

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Papaver fugax growing on the shore of Lake Kazenoi Am in the Checheno-Ingushskii ASSR at the boundary with the Dagestan ASSR has yielded 0.39% of alkaloids. Seven alkaloids have been isolated, of which cheilanthifoline, scoulerine, and reticuline have been detected in this species for the first time, while it is the first time that d-remeroline has been found in a plant of the Papaver genus.

Papaver fugax Poir. (Papaver caucasicum Bieb.) from the family Papaveraceae, Meconidium Spach (Miltantha Bernh.) section, is found in the USSR flora only in the Caucasus (Ciscuasia, Dagestan, East and South Transcaucasia [1, 2]) but it also grows in Turkey and Iran [3, 4].

Information on the alkaloid composition of P. fugax is fairly contradictory, which indicates a certain variability of the alkaloids present in it. Already, more than 23 alkaloids isolated from various samples of this species abroad [5-7] and also in our country [8] are known.

We have investigated P. fugax collected in the fruit-bearing period (September 1985) on the shore of the high-mountain lake Kazenoi Am in the Checheno-Ingushskii ASSR at the boundary with the Dagestan ASSR. Ethanolic extraction gave 0.39% of total alkaloids. From the nonphenolic fraction of the total, by chromatography on a column of silica gel, remerine and mecambrine were isolated, and from the phenolic fraction cheilanthifoline, scoulerine, armepavine, reticuline, and base (I). These alkaloids were identified by their spectral characteristics and also by the absence of depressions of the melting points of mixtures with authentic samples.

Base (I) was optically active. Its UV spectrum had absorption maxima at 222, 280, and 315 nm ($\log \varepsilon$ 4.25, 4.01, and 3.85). The mass spectrum showed the peaks of ions with m/z 295 (M^+), 280, 278, and 252, which are characteristic for the aporphine alkaloids [9]. The PMR spectrum contained the signals of the protons of a N-methyl group at 2.45 ppm and of a methylenedioxy group at 6.02 and 6.06 ppm in the form of one-proton doublets with J ~ 1.5 Hz. In the aromatic region of the spectrum there were a one-proton singlet at 6.61 ppm, a one-proton doublet at 7.98 ppm with J = 9 Hz, and a multiplet at 6.75-6.88 ppm corresponding to two protons. The spectral characteristics showed that base (I) belonged to the aporphine alkaloids with substituents in positions 1, 2, and 9. When the base was methylated with diazomethane, a O-methyl ether identical with isolaureline (II) was obtained [10]. Consequently, the base has structure (I) (see scheme on following page).

The alkaloid remeroline with structure (I) has previously been isolated from Roemeria refracta but its specific rotation was not determined. Thus, base (I) is d-remeroline.

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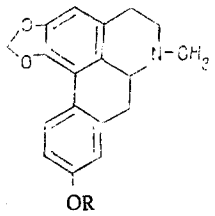

I. R = H
II. R = CH_3

EXPERIMENTAL

For chromatography we used type KSK silica gel and neutral alumina according to Brockmann (Hungary). The solvent systems 1) chloroform–ethanol (9:1) and 2) benzene–ethanol (9:1) were used for TLC. UV spectra were recorded on a Hitachi spectrometer in ethanol; PMR spectra on a JNM-100/100 MHz instrument (0 – HMDS, in $CDCl_3$, δ scale; mass spectra on a MKh-1303 mass spectrometer; and IR spectra on a UR-20 spectrometer (tablets with KBr).

Isolation and Separation of the Alkaloids. The air-dry plant P. fugax (1700 g) was extracted with ethanol. Eight extractions were made. The ethanolic extract was concentrated to a resinous mass and was then triturated with a 3% solution of acetic acid. The acid solution was washed with ether and, with cooling, was made alkaline with 25% ammonia solution, and the alkaloids were extracted successively with ether and with chloroform. The chloroform extract was evaporated to dryness. This gave 1.47 g (0.09%) of total chloroform-extracted alkaloids. The ethereal extracts, after concentration, were treated with 4% caustic potash solution. The ethereal layer was dried with anhydrous sodium sulfate and evaporated. This gave 2.29 g (0.13%) of total ether-extracted nonphenolic material.

The alkaline solution was acidified to pH 5.0-6.0 and was then made alkaline with 25% ammonia solution and shaken with ether. Evaporation of the solvent yielded 2.95 g (0.17%) of total phenolic material.

The combined ether-extracted nonphenolic alkaloids (2.29 g) were chromatographed on a column of silica gel (1:30). The alkaloids were eluted with chloroform–ethanol (99:1). The first 20 fractions (25 ml each) were combined, and this part of the total alkaloids was rechromatographed on a column of alumina. Elution with benzene–ethanol (98:2) gave 0.187 g of remerine.

Continuing elution with the same solvent mixture, 0.028 g of remeroline was isolated. R_f 0.5 in system 1 and 0.3 in system 2. mp 226-227°C, $[\alpha]_D$ +48° (c 0.39; ethanol).

Methylation of Remeroline. An ethereal solution of diazomethane was added to 10 mg of remeroline in 2 ml of absolute methanol. After a day the mixture was evaporated to dryness. A product identical with isolaureline (TLC, IR spectrum) was obtained.

On continuing elution with the same mixture, 0.32 g of mecambrine was isolated.

The total ether-extracted phenolic material (2.95 g) was chromatographed on a column of silica gel (1:30). Benzene–ethanol in various ratios was used as eluents. The fractions obtained by elution with the (99:1) mixture yielded 0.03 g of cheilanthifoline and 0.01 g of scoulerine, and the fractions obtained by elution with the (96:4) mixture gave 2.07 g of armepavine and 0.28 g of reticuline.

The combined chloroform-extracted material was chromatographed on a column of silica gel (1:30). Elution with chloroform–ethanol (99:1) yielded an additional 0.28 g of remerine.

CONCLUSIONS

The alkaloid composition of the Checheno-Ingushskii population of P. fugax has been studied. Seven alkaloids have been isolated, of which cheilanthifoline, scoulerine, and reticuline have been detected in this species for the first time, and d-remeroline has been found in the genus Papaver for the first time.

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IMMOBILIZATION OF THE ENZYME E. coli L-ASPARAGINASE ON A WATER-SOLUBLE COPOLYMER OF VINYLPYRROLIDONE AND ACROLEIN

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A method for immobilizing E. coli L-asparaginase on a copolymer of vinylpyrrolidone and acrolein has been developed and optimized. The influence on the nature of the modification of the number of acrolein residues in the copolymer has been established. The enzymatic and some physicochemical properties of the immobilized forms of the enzyme obtained have been studied.

We have previously reported the immobilization of the enzyme E. coli L-asparaginase, which is used in the treatment of a number of malignant systemic diseases of the blood, on various water-soluble polysaccharide carriers [1-3]. We now give the results of the immobilization of L-asparaginase on a synthetic water-soluble copolymer of vinylpyrrolidone and acrolein (CVA).

The interaction of L-asparaginase with the copolymer was based on the formation of an azomethine bond between the carbonyl groups of the CVA and the amino groups of the enzyme, as has been shown for the case of the modification of Terrilitin by this polymer [4]. To achieve stable links between the enzyme and the CVA, the reaction products were reduced with sodium tetrahydroborate, the free aldehyde groups of the polymer also being reduced by this reagent, as has been shown [5], and this, in the final account, imparted a neutral character to the carrier. In order to vary the electrochemical nature of the latter and to study its influence on the enzymatic activity, in individual cases reduction was also carried out with sodium bisulfite. This permitted the introduction of an additional negative charge into the polymer—protein conjugate [5].

The search for optimum conditions of synthesis ensuring the complete binding of the L-asparaginase to the CVA with the retention of a sufficiently high enzymatic activity revealed the importance of the pH of the medium and the length of the process of immobilizing the enzyme on the copolymer. Thus, the best results were obtained at pH $\geq$ 8 and a time of interaction of 3 h, i.e., under the conditions when the maximum amount of nonprotonated ε-amino groups of the lysine residues taking part in the reaction is probable.

The amount of CVA in the reaction mixture, i.e., the initial ratio of enzyme and polymer, also had a definite influence. The latter factor, in its turn, depended on the amount of acrolein residues in the samples of CVA.

The binding of L-asparaginase to the CVA led to an increase in the molecular weight of the enzyme. This was expressed in the chromatographic elution on Sephadex G-200 of the im-

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