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DYNAMICS OF THE ACCUMULATION OF ALKALOIDS IN THE EPIGEAL PART OF *Aconitum leucostomum*

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The dynamics of the accumulation of alkaloids in the epigeal part of *Aconitum leucostromum* Worosch. from various growth sites according to the vegetation periods have been studied. It has been shown that in all cases in the early period of development of the plant diterpene bases predominate in the total material, and in the fruit-bearing period isoquinoline bases.

Continuing a study of *Aconitum leucostromum* Worosch. [1], we have investigated the alkaloid composition of its epigeal part from various growth sites according to vegetation periods. Let us consider a sample collected in the Santash Pass, Terskei-Alatau range. With the growth of the plant, the amount of combined alkaloids fell sharply (%):

Vegetation period and data of collection (1977)	Total alkaloids on the weight of the air-dry plant	Diterpene alkaloids	Isoquinoline alkaloids
Rosette leaf phase, May 12	0.87	82	18
Budding, June 30	0.30	76	21
Flowering, July 10	0.18	68	30
Fruit-bearing, August 20	0.09	35	57

When the combined alkaloids were separated and their qualitative and quantitative composition was studied by the GLC method it was established that in the early vegetation period the combined alkaloids consisted mainly of diterpene derivatives and in the later periods the isoquinoline alkaloids became predominating in amount.

In the early vegetation period the plant contained lappaconitrine [2], lappaconidine [3], and an alkaloid with the composition $C_{20}H_{29}N_4$ (I) with mp 140-150°C, which, on the basis of spectral characteristics, and also by a direct comparison with an authentic sample, was identified as the aporphine alkaloid corydine [4].

In the budding period we isolated the following alkaloids: lappaconitine, corydine, lappaconidine, an unidentified base with the composition $C_{32}H_{44}N_2O_9$ (II), and a substance with the composition $C_{20}H_{25}NO_3$ (III), which was identified as the benzylisoquinoline alkaloid O-methylarmepavine [5] on the basis of the spectral characteristics and also by a direct comparison with an authentic sample.

In the flowering period, in addition to the alkaloids, isolated in the budding period, we obtained a base with the composition $C_{19}H_{23}NO_3$ (IV). The UV spectrum of the base had maxima at λ_{max} 223 and 284 nm (log ε 4.20, 3.69). In the mass spectrum of (IV) the peaks of ions with m/e 192 (100%) and 121 (25%) were observed, which are characteristic for benzyltetrahydroisoquinoline alkaloids with methoxy and hydroxy groups in the isoquinoline and a methoxy group in the benzyl parts of the molecule [6].

The NMR spectrum of (V) contained the signals of the protons of a N-methyl group (2.38 ppm, 3 H, singlet), and of two methoxy groups (3.63 and 3.78 ppm, 3 H each, singlets). Two-proton doublets at 6.37 and 6.91 ppm (J = 8 Hz) were assigned to two equivalent pairs of ortho

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protons in the benzyl part of the molecule, and singlets at 6.4 ppm, 1 H, and 6.25 ppm, 1 H relate to the C_5-H and C_8-H atoms, respectively. A downfield shift of the C_8-H signal shows the presence of a hydroxy group at C_7 [7].

The facts given enabled the base to be identified as N-demethylcolletine [8].

In the fruit-bearing period we detected chromatographically lappaconidine and succeeded in isolating lappaconitine, corydine, O-methylarmepavine, N-demethylcolletine, and alkaloids with the compositions $C_{32}H_{44}N_2O_9$ (II) and $C_{20}H_{21}NO_5$ (V). On the basis of spectral characteristics and also a direct comparison with an authentic sample, the latter was identified as the aporphine alkaloid glaunidine [9].

We studied the alkaloid compositions of the epigeal parts of *A. leucostomum* from different growth sites. By separating the combined alkaloids and also by a GLC study we found the ratio of the diterpene and isoquinoline alkaloids in them. The amounts of alkaloids in *A. leucostomum* from different growth sites in the rosette leaf phase were as follows (%):

Site and data of collection (1979)	Total amount of alkaloids on the weight of the air-dry plant	Diterpene alkaloids	Isoquinoline alkaloids
Valley of the R. Karkara, May 29	0.51	64	36
Ketmen' range (Tuyuk), June 3	0.25	90	10
Terskei-Alatau range (Sarydzhas), June 4	0.33	98	2
Kungei-Alatau range (Saty), June 5	0.37	83	17
Dzhungarian Alatau range (Sarybel'), June 9	0.33	89	11
Kirghiz range (Tyuzashu), June 10	0.30	94	4
Ketmen' range (Uzun-Bulak), June 13	0.46	63	34
Terskei-Alatau range (Aksu), June 15	0.28	96	3

There is information to the effect that the families Ranunculaceae and Papaveraceae are directly related in the phylogenetic respect [10]. In actual fact, magnoflorine has been isolated from *Aconitum* sp. [11], higenamine (dl-demethylcoclaurine) from *Aconitum japonicum* Thumb. [12], isoboldine from *A. karakolicum*, and delporphine, N-methyllaurotetanine, and isoboldine from *Delphinium dictyocarpum* [14].

We have observed an unusual phenomenon: In the fruit-bearing stage isoquinoline bases predominate in the total alkaloids of the epigeal part of *A. leucostomum*.

EXPERIMENTAL

Melting points are uncorrected. NMR spectra were taken on JNM 100/100 MHz and C-60-HL instruments in deuterochloroform with MDS as internal standard (the figures are given in the δ scale); mass spectra were taken on MKh-1310 and MKh-1303 instruments fitted with systems for direct introduction into the ion source; IR spectra in KBr tablets and in chloroform; and UV spectra in ethanol on a Hitachi spectrometer. GLC was performed on a DIP instrument using a column containing 10% of OV-1 on Chromosorb W, 60/80 mesh, 1.52 × 3.18 mm, T_{det} = 330°C, T_{evap} = 340°C, T_{col} = 180–320°C (V_{heat} = 5 deg/min), $V_{He}/V_{H_2}/H_{air}$ = 15/30/300 ml/min.

For chromatography we used type SKS silica gel and alumina (Brockmann activity grade II).

Isolation and Separation of the Combined Alkaloids. The air-dry plant material collected in the rosette-leaf phase (1.7 kg) was moistened with 5% sodium carbonate solution and exhaustively extracted with chloroform. The chloroform extract was shaken with 5% sulfuric acid, and the acid solution was washed with chloroform and then, with cooling, it was made alkaline with sodium carbonate and alkaloids were extracted with chloroform. This gave 14.71 g of combined alkaloids. Of this material, 3 g was dissolved in ethanol and 20% hydrobromic acid was added to give an acid medium, whereupon 1.81 g of lappaconitine hydrobromide was ob-

tained. The residual mother liquor was chromatographed on a column of alumina and elution with benzene yielded 0.5 g of lappaconitine and with a mixture of benzene and methanol 0.02 g of corydine and 0.01 g of lappaconidine.

By the method described above, from 1.5 kg of plant material collected in the budding period we obtained 4.5 g of the combined alkaloids which were dissolved in 5% sulfuric acid and, after the alkaloids had been liberated by alkalinization, they were extracted successively with hexane, ether, and chloroform. The hexane fraction obtained amounted to 0.7 g, the ether fraction to 2.6 g, and the chloroform fraction to 0.8 g. When the hexane fraction was treated with acetone, 0.2 g of lappaconitine separated out, and the ether fraction by similar treatment gave 0.9 g. The remaining mother liquor was chromatographed on a column of alumina and 0.2 g of lappaconitine, 0.08 g of corydine, and 0.02 g of O-methylarmepavine were isolate-

The chloroform-extracted combined alkaloids of *A. leucostromum* (0.8 g) were chromatographed on a column of alumina. Benzene–methanol eluates gave 0.1 g of lappaconitine, 0.02 g of corydine, 0.03 g of a base with the composition $C_{32}H_{44}N_2O_9$ (II), and 0.3 g of lappaconidine.

From 0.9 kg of the plant collected in the flowering period we obtained 3.34 g of combined alkaloids, which were chromatographed on a column of silica gel. When the alkaloids were eluted with chloroform, 0.3 g of lappaconitine, 0.15 g of base (II), and 0.18 g of corydine were obtained. Rechromatography of the mother liquors on a column of alumina yielded 0.05 g of lappaconitine and 0.04 g of O-methylarmepavine, and on a column of silica gel 0.006 g of corydine and 0.02 g of N-demethylcolletine.

From 2.4 kg of plant material collected in the fruit-bearing period we obtained 2.6 g of total alkaloids, which were chromatographed on a column of alumina. Elution of the alkaloids with benzene gave 0.05 g of O-methylarmepavine, 0.03 of lappaconitine, and 0.05 g of corydine, and then benzene–methanol gave 0.009 g of corydine and 0.021 g of glaunidine. Rechromatography of the mother liquor on a column of silica gel and elution with chloroform gave 0.08 g of lappaconitine, 0.07 g of corydine, and 0.04 g of N-demethylcolletine.

In all the combined diterpene alkaloids of the plants collected from different growth sites, lappaconitine was present in the largest amount (see above).

Methylation of N-Demethylcolletine (IV). A mixture of 20 mg of the base (V), 1 ml of absolute ethanol, and an ethereal solution of diazomethane was left for three days. The solvent was evaporated off, giving O-methylarmepavine.

Preparation of a Sample for GLC. The combined alkaloids (50 mg) were boiled in a 5% solution of NaOH in methanol for 1.5 h, and the solvent was evaporated off. The residue was dissolved in water and extracted with chloroform, the solvent was distilled off, and the residue was dissolved in ethanol.

SUMMARY

The dynamics of the accumulation of alkaloids in the epigeal part of *Aconitum leucostrom* Worosch. collected in the Santash pass, Terskei-Alatau range with respect to vegetations period, and of the combined alkaloids of plant from different growth sites has been studied. Separation of the combined alkaloids yielded, in addition to the lappaconitine and lappaconidine described previously, the isoquinoline alkaloids corydine, O-methylarmepavine, N-demethyl colletine, and glaunidine, which have not previously been isolated from plants of the genera *Aconitum* and *Delphinium*, and an unidentified diterpene base $C_{32}H_{44}N_2O_9$. It has been shown that in the fruit-bearing period the isoquinoline alkaloids predominate in the combined alkaloids.

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DENATURATION AND RENATURATION OF THE 11S GLOBULIN OF COTTON SEEDS BY POLAROGRAPHY

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It has been established by a polarographic analysis of the globulins of cotton seeds that the 7S and 11S golublins possess a two-step polarographic wave with a half-wave potential of —1.42 V. On the basis of the results of a study of the kinetics of thermal denaturation the high lability of the 11S globulin on heating has been shown. The conditions have been determined of the complete denaturation of the 11S globulin in 8 M urea solution and it has been established that the latter is an irreversible process.

The total globulin fraction of cotton seeds consists mainly of two components with sedimentation coefficients of 7S and 11S and molecular weights of 130,000 [1], and 280,000 [2], respectively, which have complex quaternary structures [3, 4]. In a study of the physicochemical properties of the proteins isolated we have established that they possess a two-step polarographic wave and have the same half-wave potential of —1.42 V (Fig. 1).

An investigation of proteins not possessing a quaternary structure has shown that in the process of catalytic reduction of hydrogen ions and the formation of the second step of the wave the main role is played by SH groups and S—S bridges [5]. In the present case, the 11S

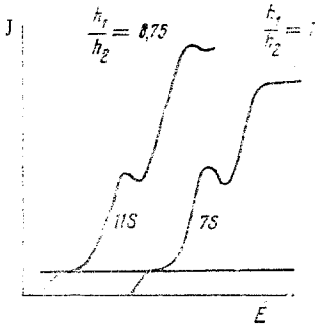

Fig. 1

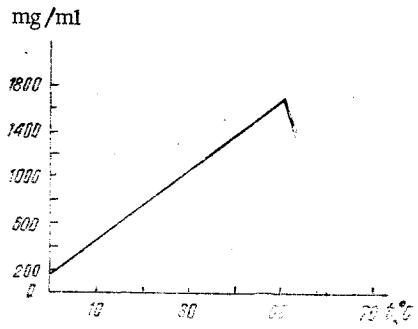

Fig. 2

Fig. 1. Two-step polarographic wave of the native 7S and 11S globulins.

Fig. 2. Temperature dependence of the solubility of the 11S globulin.

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