9. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).
10. W. Klyne, Biochem. J., 47, No. 4, xli (1950).

ALKALOIDS OF THE EPIGEAL PART OF Aconitum karakolicum

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The dynamics of the accumulation of alkaloids in the epigeal part of Aconitum karakolicum according to vegetation periods has been studied. It has been established that the highest content of alkaloids is found in the early period. On separating the combined alkaloids, the aporphine alkaloid isoboldine has been isolated from this plant for the first time and a new base has been obtained — napelline N-oxide, the structure of which has been shown on the basis of the results of a study of spectral characteristics and direct transition to napelline.

Results have been given previously of a study of the alkaloids of the epigeal part of Aconitum karakolicum [1]. Attention was then directed to the fact that in the epigeal part of the plant the highly toxic aconitine is not the main component of the mixture of alkaloids, but the dominating alkaloids are those of the songorine group: napelline, acetylnapelline, and songorine itself [1, 2]. In view of the fact that the alkaloids of this group are of definite interest from the point of view of their physiological activity, we have studied the alkaloids of the epigeal part of Aconitum karakolicum collected in the upper reaches of R. Tyup (Kirghiz SSR) in various vegetation periods. Below we give information on the quantitative content of the total alkaloids (in percentages of the weight of the dry plant):

Vegetation Period and Date of Collection (1977)	Total Alkaloids, %
Early period, May 12	1.14
Budding, July 10	0.26
Flowering, August 5	0.22
Ripening of the seeds, August 25	0.17

The separation of the total alkaloids showed that in the early period the plant contains aconitine, aconifine, acetylnapelline, songorine, napelline, a base with mp 122-124°C having the composition $C_{19}H_{21}NO_4$, and a base with mp 197-199°C having the composition $C_{22}H_{23}NO_4$. On the basis of the results of a study of spectral characteristics, and also by direct comparison with an authentic sample, the base with mp 122-124°C was identified as the aporphine alkaloid isoboldine [3-5].

The alkaloid with mp 197-199°C was readily soluble in water and methanol, moderately soluble in chloroform and acetone, and sparingly soluble in ether. The NMR spectrum of the base showed the following signals: a three-proton singlet at 0.80 ppm ($>C{-}CH_3$), a three-proton triplet at 1.32 ppm ($N{-}C_2H_5$), and poorly resolved one-proton doublets at 5.09 and 5.25 ppm (terminal methylene group). The spectral characteristics of the alkaloid are close to those for napelline. The molecular weight of the base was 16 m.u. greater than that of napelline. This difference, and also the good solubility of the alkaloid in water and the presence in its mass spectrum of a characteristic triplet of peaks $M^+ - 16$, $M - 17$, and $M - 18$ permitted the assumption that the base was napelline N-oxide (I). In actual fact, when compound (I) was reduced with ferrous sulfate a product was obtained which was identified as napelline.

On separating the total alkaloids obtained in the budding period, we isolated the same alkaloids as in the earlier period, with the exception of aconifine.

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In the fruit-bearing period, acetylnapelline and napelline were detected chromatographically, while it was possible to isolate aconitine, songorine, napelline N-oxide, and isoboldine. The largest amount of napelline, acetylnapelline, and songorine was observed in the early period. At the same time, as the plant developed, the amount of napelline N-oxide increased with a simultaneous decrease in the amount of napelline itself.

EXPERIMENTAL

Melting points are uncorrected. The NMR spectra were taken on a JNM-4H-100/100 MHz instrument in deuteromethanol and deuterochloroform with HMDS as internal standard (the values are given in the δ scale), and the mass spectra on MKh-1310 and MKh-1303 instruments fitted with systems for direct introduction into the ion source. Type KSK silica gel and alumina (Brockmann activity grade II, neutral) were used for chromatography.

Isolation and Separation of the Total Alkaloids. The air-dry plant collected in the early vegetation period (1280 g) was moistened with a 5% solution of sodium carbonate and extracted with chloroform. The chloroform extract was shaken with 5% sulfuric acid; the acid solution was made alkaline with sodium carbonate, with cooling, and it was extracted first with ether and then with chloroform. Seven extracts were made, and distillation of the ether yielded 13.93 g of combined alkaloids while distillation of the chloroform gave 0.67 g of combined alkaloids.

The ethereal fraction of the total alkaloids (9.69 g) was separated according to basicity into 13 fractions. The acetone treatment of fractions 1-2 led to the separation of 0.24 g of aconitine, while fractions 3-6 gave 1.09 g of acetylnapelline. The mother liquor from fractions 3-6 was evaporated to dryness, the residue was dissolved in methanol, an alkaline solution of hydrochloric acid was added to a weakly acid reaction, and 0.57 g of songorine hydrochloride was isolated. The action of ethanolic hydrochloric acid on fractions 7-9 yielded an additional 0.82 g of songorine hydrochloride. By the action of acetone, fractions 10 and 11 yielded 0.76 g of napelline. When fraction 13 was treated with acetone 0.64 g of a powder was obtained which was chromatographed on a column of alumina. Elution was carried out with chloroform to which gradually increasing amounts of methanol were added. On elution with chloroform–methanol (25:1), with the aid of chloroform 0.23 g of a base with mp 197-199°C (napelline N-oxide) was obtained.

Napelline N-Oxide. IR spectrum: 3400-3500 cm^{-1} (OH group). NMR spectrum, ppm: 0.80 (3 H, singlet), 1.32 (3 H, triplet), 5.09 and 5.25 (1 H, doublets, J = 1.5 Hz). Mass spectrum: M^+ 375.

Reduction of Napelline N-Oxide. A solution of 0.09 g of napelline N-oxide in 4 ml of water was shaken with 0.3 g of $FeSO_4$ for 9 h. The aqueous solution was acidified with 5% sulfuric acid and was washed three times with ether, and it was then made alkaline with ammonia and was extracted with ether. The extract was dried over sodium sulfate and distilled. After elimination of the solvent, with the aid of acetone 0.025 g of napelline was isolated.

The mother liquors from fractions 1-6 were combined and chromatographed on a column of alumina. On elution with chloroform, fractions 7-13 yielded 0.78 g of acetylnapelline, and elution with chloroform–methanol (50:1) gave 0.77 g of napelline. With the aid of methanol, the chloroform–methanol (25:1) eluates yielded 0.03 g of a base with mp 122-124°C, which was identified as isoboldine on the basis of a mixed melting point with an authentic sample and the results of a study of their spectra.

By the method described above, 1397 g of the plant collected in the budding period yielded 2.68 g of total alkaloids.

The total alkaloids were separated according to basicity into nine fractions. With the aid of acetone, fractions 2-3 yielded 0.04 g of aconitine, and fractions 4-6 gave 0.41 g of acetylnapelline. All the fractions were combined and were saponified with a 5% solution of KOH in methanol, the methanol was evaporated off, and the residue was dissolved in water and exhaustively extracted with chloroform. The chloroform extract, after drying over sodium sulfate, was distilled. The combined alkaloids so obtained were chromatographed on a column of alumina. The action of an ethanolic solution of hydrogen chloride on the chloroform–methanol (50:1) eluates led to the separation of 0.33 g of songorine hydrochloride, and elution with chloroform–methanol (35:1) led to the isolation of 0.24 g of napelline. Elution with chloroform–methanol (25:1) gave 0.009 g of isoboldine, and the chloroform–methanol (1:1) eluates gave 0.12 g of napelline N-oxide.

From 400 g of the plant collected in the flowering period we obtained 0.88 g of total alkaloids.

The total alkaloids were separated on a column of alumina. Elution was carried out with benzene to which gradually increasing amounts of methanol were added. Benzene–methanol (25:1) led to the isolation of 0.006 g of isoboldine, and benzene–methanol (15:1 and 10:1) gave 0.03 g of napelline N-oxide. All the preceding fractions were combined and rechromatographed on a column of alumina. Chloroform–methanol (100:1) eluates gave 0.004 g of aconitine. On elution with a mixture of chloroform and methanol (50:1), fractions 1-9 furnished 0.11 g of acetylnapelline, and the action of an ethanolic solution of hydrochloride on fractions 13-19 yielded 0.09 g of songorine hydrochloride. Elution with chloroform–methanol (25:1 and 1:1) gave an additional 0.04 g of napelline N-oxide.

From 1537 g of plant collected in the period of the ripening of the seeds, we obtained 2.61 g of total alkaloids.

The total alkaloids were chromatographed on a column of alumina. Elution was carried out first with benzene, and with the aid of acetone fractions 3-9 yielded 0.007 g of aconitine. By the action of an ethanolic solution of hydrochloric acid, benzene–methanol (100:1 and 50:1) eluates gave 0.34 g of songorine hydrochloride, and elution with benzene–methanol (25:1) and with methanol gave 0.43 g of napelline N-oxide.

SUMMARY

The dynamics of the accumulation of alkaloids in the epigeal part of Aconitum karakolicum collected in the upper reaches of the R. Tyul according to vegetation periods has been studied. Separation of the total alkaloids has yielded the aporphine alkaloid isoboldine, which has not been isolated from this plant previously, and the new alkaloid napelline N-oxide, the structure of which has been shown on the basis of the results of a study of spectral characteristics and by direct conversion into napelline.

LITERATURE CITED

1. M. N. Sultankhodzhaev, L. V. Beshitaishvili, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 681 (1976).
2. M. N. Sultankhodzhaev, L. V. Beshitaishvili, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prir. Soedin., 479 (1978).
3. H. Chikamatsu, M. Tomita, and M. Kotake, J. Chem. Soc. Jpn., 82, 1708 (1961).
4. L. D. Yakhontova, in: Medicinal Plants [in Russian], Vol. XV, Moscow (1969), p. 348.
5. A. Kh. Yunusov, I. A. Israilov, and M. S. Yunusov, Khim. Prir. Soedin., 681 (1973).