The chemical properties of nitraramine have been studied. The possibility has been established of the existence of the product of its hydrolysis in three tautomeric forms: an amino aldehyde chain form and two cyclic forms — α-carbinolamine and semiacetal.

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# ALKALOIDS OF THE ROOTS OF THE *Haplophyllum obtusifolium*


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The roots of the *Haplophyllum obtusifolium* Ledeb. have yielded robustine, dictamnine, skimmianine, γ-fagarine, and evoxine and the new alkaloid haplobine for which on the basis of spectral characteristics and a passage to the main alkaloid haplopine (7-hydroxy-4,8-dimethoxyfuranoquinoline) the structure of 7-(3'-chloromethylbut-2'-enyloxy)-4,8-dimethoxyfuranoquinoline has been established.


The isolation from the epigeal part of the *Haplophyllum obtusifolium* Ledeb. collected in the environs of Mt. Nukus of evoxine, haplopine, acetylhaplatine, skimmianine, evodine, and methylevoxine has been reported previously [1]. The present paper is devoted to a study of the alkaloids of the roots of *H. obtusifolium* from the same growth site.

The roots were extracted with methanol. The evaporated extract was separated in the usual way into basic and neutral fractions. Chromatography of the basic fractions yielded robustine, dictamnine, base (I), skimmianine, γ-fagarine, and evoxine.

Base (I) had mp 151-153°C. Its mass spectrum permitted the assumption that its molecule contained a chlorine atom, since in the region of the molecular ion there were two peaks, with m/z 347 (19%) and 349 (6.2%) differing by two units and with an intensity ratio of 3:1, which is characteristic for chlorine-containing compounds [2]. The spectrum also contained the peaks of the M − 35 ion (m/z 312) formed as the result of the cleavage of a C—Cl bond.

The presence of a chlorine atom was confirmed by the Beilstein test and Stepanov's qualitative reaction [3]. Base (I) is new and we have called it haplobine.

The UV spectrum of (I) coincided with that of furanoquinoline alkaloids with alkoxy substituents in positions 4, 7, and 8, for which maxima at 250, 320, and 335 nm and a deep minimum in the 280 nm region are characteristic [4]. The IR spectrum had absorption bands at 3115 and 3145 $cm^{-1}$ (unsubstituted furan ring). There was no absorption due to active hydrogen.


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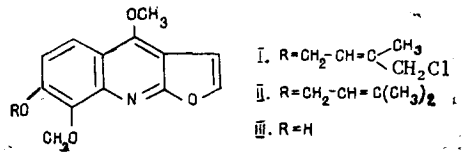
The facts given above, and also the almost complete analogy of the mass spectrum of haplobine with those of the alkaloids 7-isopentenyloxy-γ-fagarine (II) [5] and haplopine (III), beginning from the m/z 245 fragment (for (III) this is the $M^+$ ion and for (I) and (II) the $(M - R)^+$ fragment formed as the result of the splitting out of the side chains with the migration of a hydrogen atom), permitted the assumption that the base isolated was 4,8-dimethoxyfuranoquinoline with a $O-C_5H_8Cl$ substituent in position 7. A confirmation of this was the formation of the phenolic alkaloid (III) when haplobine was hydrolyzed in an acid medium. The ease of hydrolysis of (I) also showed the allyl position of the double bond in relation to the oxygen atom in the side chain.

The structure of the chlorine-containing allyloxy substituent followed unambiguously from the PMR spectrum of haplobine, in which there were signals with the following δ values (ppm): 7.93, 7.15 and 7.53, 6.99 (two pairs of doublets, one H each, J = 9.4 Hz and 2.8 Hz; ortho-aromatic protons and the protons of a furan ring); 5.68 and 4.78 (triplet, 1 H, and doublet, 2 H, J = 7 Hz; $O-CH_2-CH=$); 4.35 and 4.05 (singlets, 3 H each, 2 $ArOCH_3$); and 4.09 and 1.87 (singlets, 2 H and 3 H, $=C(CH_3)(CH_2Cl)$).

Thus, haplobine has the structure of 7-(3'-chloromethylbut-2'-enloxy)-4,8-dimethoxyfuranoquinoline.




Halogenated alkaloids are rarely found in plants. There is information in the literature on the isolation from the roots of *Ruta graveolens* of chlorine-containing acridone alkaloids [6] which accompany quinoline alkaloids in plants of the family *Rutaceae*. However, this is the first time that a chlorine-containing compound has been detected among the quinoline alkaloids of this family.

## EXPERIMENTAL

The spectra of the substances were obtained on the following instruments: Hitachi EPS-3T (ethanol); UR-20 (KBr); MKh-1310; and BS-567A, 100 MHz; (δ scale, $CDCl_3$, HMDS).

Isolation of the Alkaloids. The dry comminuted roots (980 g) were extracted with methanol. After the methanol had been driven off, the extract was divided into basic (2.16 g) and neutral (18 g) fractions. The basic fraction was chromatographed on silica gel L 100/250 μ (Czechoslovakia). Ethereal eluates yielded substances in the following sequence: robustine, dictamnine, haplobine, skimmianine, γ-fagarine, and evoxine.

Chromatographic monitoring was carried out by TLC (silica gel 5/40 μ) in the following solvent systems: 1) toluene–ethyl acetate–formic acid–methanol (5:4:1:1); 2) ethyl acetate; and 3) ether.

Haplobine (I), mp 151-153°C (from acetone). UV spectrum: $\lambda_{max}$ 251, 319, 330 nm (log ε 4.73, 3.60, 3.67); $\lambda_{min}$ 280 nm (log ε 3.30). Mass spectrum: m/z (%): 349 (6.2), 347 ($M^+$, 19), 312 (4), 245 (50), 244 (100), 230 (10), 227 (43), 216 (31), 199 (9).

Hydrolysis of Haplobine. A solution of haplobine (3 mg) in concentrated hydrochloric acid (1 ml) was heated in the water bath for 5 min. The acid solution was made alkaline with ammonia and extracted with chloroform. The chloroform extract was chromatographed on a column of silica gel. This gave crystals identical according to a mixed melting point and TLC in systems 1-3 with a sample of haplopine (III). Mass spectrum of (III): m/z (%): 245 ($M^+$, 100), 244 (22), 230 (28), 227 (78), 216 (17), 199 (22).

## SUMMARY

The roots of the plant *Haplophyllum obtusifolium* have yielded robustine, dictamnine, skimmianine, γ-fagarine, and evoxine and the new alkaloid haplobine, for which the structure of 7-(3'-chloromethylbut-2'-enyloxy)-4,8-dimethoxyfuranoquinoline has been established.

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# PROTEASE A FROM COTTON SEEDS.

# ISOLATION AND PURIFICATION OF THE ENZYME

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Protease A has been isolated in the homogeneous state from dormant seeds of cotton plants of the Tashkent-I variety. A scheme is proposed for the isolation and purification of the enzyme which includes the following stages: extraction of the defatted seeds with 0.1 M phosphate buffer, pH 7.4; precipitation of the protein with ammonium sulfate at 60% saturation; desalting by dialysis; and ion-exchange chromatography on a column containing CM- and DEAE-celluloses. The molecular weight of the enzyme has been determined as 60,000. The enzyme efficiently hydrolyzes azocasein and the 7S and 11S reserve proteins of cotton seeds. Its maximum activity appears at pH 6.4-7.4 and a temperature of 35-40°C; it is not activated by sulfhydryl reagents and loses its activity in the presence of diisopropyl phosphorofluoridate. The assumption is made that protease A belongs to the serine type of trypsin-like proteases.

The presence of proteolytic enzymes in cotton seeds was first reported by Rossi-Fanelli et al. [1]. Then Gatsu and Jacks detected the presence of a proteinase with an acid pH optimum of its action in cotton seeds in a form associated with the aleurone grains [2]. On the basis of this fact, it was assumed that these enzymes played an important role in the breakdown of the reserve protein during the growth of the seeds.

In investigations of the proteolytic enzymes of dormant cotton seeds performed previously, we showed that 75% of the total activity is due to acid proteases [3] and their molecular weights are between 30,000 and 140,000 [4]. In the present paper we give the results of the isolation and purification of a homogeneous proteolytic enzyme of cotton seeds — protease A [5]. A scheme has been developed for purifying protease A which includes the following steps: extraction of the defatted seeds with 0.1 M phosphate buffer, pH 7.4; precipitation of the protein with ammonium sulfate at 60% saturation; desalting by dialysis; and ion-exchange chromatography on a column containing CM- and DEAE-celluloses. Information on the isolation and purification procedure is given below: (following page, below Fig. 1).

The homogeneity of the purified enzyme was established by electrophoresis in polyacrylamide gel and by the method of isoelectric focussing in a borate-polyol system by the method described in [6]. The isoelectric point of protease A is located in the region of pH 4.9. Graphs of the purification of protease A on columns containing CM- and DEAE-celluloses are given in Figs. 1, 2, and 3. The molecular weight of the enzyme was determined by its gel filtration through a column of Sephadex G-200 that had been calibrated with protein markers

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