AN INVESTIGATION OF THE ALKALOIDS OF *Thermopsis lanceolata*

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The quantitative and qualitative composition of the alkaloids in plants of the genus Thermopsis depends markedly on the vegetation period [1]. We have begun to investigate the epigeal part of the plant collected in the environs of the village of Ulakhol, Kirgiz SSR (southern shores of Lake Issyk-Kul') at the beginning of the vegetation period. A distinguishing feature of this plant is its high content of extractive substances which does not permit it to be extracted with water using cation-exchange resins [2].

Chloroform extraction yielded 4.14% of combined alkaloids. Repurification showed that more than 1% consisted of substances of nonalkaloid nature. The alkaloids present in largest amount are thermopsine, cytisine, pachycarpine, and N-methylcytisine. In order to isolate these components, the combined alkaloids were separated into ethereal, benzene, and chloroform fractions.

The pachycarpine passed into the ether, from which it was readily precipitated in the form of the perchlorate. The total amount of pachycarpine in the combined alkaloids was 14%.

The thermopsine (35%) was also obtained from the ethereal fraction, by concentration. The cytisine was found mainly in the benzene and chloroform. To determine the amount of cytisine present, part of the combined alkaloids was separated by a published method [3] and the yield of cytisine was found (30% of the total).

The ethereal fraction was separated in detail. After the main alkaloids had been removed, the mother liquor yielded N-methylcytisine and a liquid base with the composition $C_{15}H_{20}N_2O$, optically active, giving a crystalline perchlorate and picrate. A study of its IR, mass, and NMR spectra, and also the results of a comparison of its physicochemical properties showed that the alkaloid was similar to rhombifoline [4]. Because we had no authentic sample and some of the constants did not agree with those given in the literature, it was impossible to identify the substance definitively. Consequently, we prepared the dihydro base and compared it with the N-butylcytisine from cytisine. By determining the melting points of mixtures and by comparing the IR spectra, the perchlorates were shown to be identical.

When the components of the ethereal mother solution were separated with respect to their basicities, the strongly basic fraction yielded two crystalline bases with mp 235 and 154-155° C. The latter was optically active and had the composition $C_{15}H_{26}N_2O$ (both nitrogen atoms being tertiary) and gave a crystalline methiodide and dipicrate. Since the given constants differed from those of known alkaloids, we have called it thermopsamine. In order to determine the nature of the hydroxy group, the base was subjected to Oppenauer oxidation, giving a ketone in the IR spectrum of which there was an absorption band at 1720 cm^{-1}. To establish its basic skeleton, the alkaloid was heated with phosphorus pentoxide. A mixture of three anhydro products was obtained. Although the mixture was difficult to separate and the first product eluted on chromatography on a column of alumina changed into a different compound after concentration and standing [5], nevertheless the NMR spectrum of this product exhibited signals of olefinic protons in the 5-6-ppm region. The catalytic hydrogenation of this mixture in the presence of Pt yielded sparteine and α-isosparteine. Consequently, thermopsamine is a hydroxy derivative of the sparteine series. The oxidation of the alkaloid with potassium ferricyanide formed an amide with mp 221° C; its IR spectrum exhibited absorption bands of an amide carbonyl at 1645 cm^{-1} and of a hydroxy group at 3430 cm^{-1}.

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The results of a comparison of the mass spectra of this amide and of 17-oxosparteine showed that there is a hydroxy group in ring D. The mass and IR spectra of thermopsamine and of 13-hydroxysparteine agreed completely [6, 7]. Thus, thermopsamine is the optical antipode of the 13-hydroxysparteine obtained by the reduction of hydroxylupanine.

EXPERIMENTAL

Chromatography was performed with type KSK silica gel, neutral alumina, and type M ["slow"] paper of the Volodarskii Leningrad Mill, and the following solvent systems: 1) chloroform–methanol (4 : 1), 2) chloroform–benzene–methanol (5 : 4 : 1), and 3) isobutanol–conc. HCl–water (100 : 13 : 27). The spots were revealed with Dragendorff's reagent.

The epigeal part of the plant (120 kg) was moistened with 8% ammonia and treated with chloroform until the alkaloids had been extracted completely. The extract was treated with 5% H_2SO_4. The acid solution was made alkaline with conc. ammonia and the free bases were extracted with chloroform. This gave 4.97 kg of combined alkaloids (4.14%). They were dissolved in 6 liters of 5% H_2SO_4 and the acid solution was washed with ether, made alkaline to pH ≈ 11, and exhaustively extracted with ether (2124 g), benzene (500 g), and chloroform (980 g).

Substances were detected with R_f (system 1) 0.05, 0.3, 0.7, 0.81, 0.87, and 0.92 (ethereal fraction), 0.05, 0.3, 0.69, (max), and 0.82 (benzene fraction), and 0.3 (max), 0.69, 0.82 and 0.87 (chloroform fraction).

Thermopsine. When the ethereal extract was concentrated and the residue was triturated with acetone, 443 g of thermopsine with mp 203-206° C was obtained.

Pachycarpine. The mother liquor after the separation of the thermopsine was mixed with ethanol, and perchloric acid was added to pH 6; the crystalline perchlorate of pachycarpine with mp 169-170° C deposited immediately (557 g). The residue from the mother liquor was recrystallized from water, giving an additional 70 g of pachycarpine perchlorate.

N-Methylcytisine and Rhombifoline. The mother liquor from the perchlorates, after the water had been driven off, was treated with acetone. This gave 620 g of a mixture of perchlorates with R_f 0.92 (min), 0.87, and 0.7 (max). This mixture could not be separated according to differences in the solubilities of the salts and the bases. Consequently, 405 g of the salts were converted into the bases (260 g) and these were dissolved in 1.3 liter of 5% H_2SO_4 and separated according to their base strengths into five fractions. Of the first fraction, 6 g was passed through a column of silica gel (180 g). The chloroform eluate yielded 0.2 g of rhombifoline in the form of viscous, rapidly darkening oil, $[\alpha]_D$ −151° (c 0.6; chloroform), R_f 0.92 (system 1). Its perchlorate had mp 232-234° C and its picrate mp 139-141° C. IR spectrum, ν_{max}, cm^{-1}: 978, 1468, 1554, 1660. Mol. wt. 244 (mass spectrometry; the 100% peak was M−41). The second to fifth fractions yielded 200 g of N-methylcytisine.

Base with mp 235° C and Thermopsamine. The alkaloids in the mother liquor from the perchlorates of the ethereal fraction, after the separation of the alkaloids mentioned above, were converted into the bases (602 g). These were dissolved in 5.5 liters of 2.5% H_2SO_4 and, with the addition of 35-ml portions of 10% caustic soda, were extracted with chloroform. A total of 31 fractions was obtained. Fractions 1-7 contained practically no alkaloids. Fractions 8-17 yielded 85 g of thermopsine perchlorate (from acetone). Fractions 18-20 gave 80 g of pachycarpine perchlorate. By the treatment of fractions 20-28 with a mixture of ether and acetone, 52 g of cytisine with mp 151-153° C was isolated. When fraction 29 was triturated with acetone, colorless crystals deposited with mp 235° C, $[\alpha]_D$ +121.8° (c 1.0; chloroform), R_f 0.3 (system 1).

Since fractions 30-31 could not be separated by means of the differences in the solubilities of the salts, they were separated into water-soluble and water-insoluble components. The aqueous solution was made alkaline and extracted with petroleum ether, ether, and chloroform. On concentration, the petroleum ether and ether fractions deposited colorless needles with mp 154-155° C, $[\alpha]_D$ +26.4° (c 0.9; ethanol), R_f 0.05 (system 1), 0.2 (system 2), and 0.4 (system 3). IR spectrum, ν_{max}, cm^{-1}: 3370 (OH group) and 2680-2800 (trans-quinolizidine). No absorption in the UV region. Mol. wt. 250 (mass spectrometry). Yield of technical thermopsamine 4 g. The substance forms a dipicrate with mp 130-131° C and a methiodide with mp 244-246° C (decomp.).

Cytisine. When the benzene fraction was concentrated and the chloroform fraction was treated with benzene and acetone, 420 g of cytisine with mp 151-153° C, R_f 0.3 (system 1), 0.23 (system 3) was isolated.

N-Butylcytisine. Rhombifoline (0.05 g) was hydrogenated in ethanol over 0.0261 g of PtO_2 for 10 min. The amount of hydrogen absorbed was 4.7 ml. The solution was separated from the catalyst. The perchlorate of the product gave no depression of the melting point with an authentic sample of N-butylcytisine.

Dehydration of Thermopsamine. Thermopsamine (0.5 g) was mixed with sand, and 8 g of P_2O_5 was added and the resulting mixture was heated at 170-180° C for 2.5 h. Then it was decomposed with ice, made alkaline, and extracted with ether. The mixture of products (0.35 g) with R_f 0.9, 0.6, 0.4, and 0.2 (initial) (system 2) was passed through Al_2O_3, and petroleum ether–ether (10 : 1) eluted (fraction 1) 40 mg of a base with R_f 0.9 which, on standing, formed a substance with R_f 0.6 (≈ 50% of the original), and then petroleum ether–ether (4 : 1 and 1 : 1) eluted (fraction 2) 0.98 mg of a mixture of bases with R_f 0.6 and 0.4 (system 2). The hydrogenation of the mixture of anhydro products formed α-isosparteine and sparteine, which were identified by means of their R_f values in three systems.

Oxidation of Thermopsamine. A solution of 100 mg of the base in 3 ml of 3% HCl was treated with 1.3 g of $K_3[Fe(CN)_6]$ and left at room temperature for 2 days. After alkalization with NaOH, the products were extracted with chloroform. The resulting amide had mp 221° C, R_f 0.6 (system 1). Yield 75%.

Oppenauer Oxidation. In a current of nitrogen at 5° C, 0.2 g of thermopsamine in 10 ml of absolute benzene was added to a mixture of 0.6 g of freshly sublimed potassium tert-butoxide, 10 ml of absolute benzene, and 0.4 g of fluorenone. The mixture was left at room temperature for 5 h and was then treated with acetic acid, made alkaline, and extracted with ether. The resulting mixture, with R_f 0.2 (initial) and 0.6 (system 2) was passed through a column of silica gel. IR spectrum of the product: 1720 cm^{-1} $\left(-C\diagdown\!\!\!\diagup^{O}\right)$.

Reduction. Red phosphorus was added to a solution of 100 mg of thermopsamine in 1 ml of acetic acid and 2 ml of HI, and the mixture was boiled in an air bath for about 40 h. Then the reaction mixture was chromatographed on paper in system 3, giving spots with R_f 0.4 and 0.67, the R_f value of the initial substance being 0.4 and that of pachycarpine 0.65.

Chlorination of Thermopsamine. With cooling to –10° C, freshly distilled $SOCl_2$ was added to a solution of 0.2 g of the base in 2 ml of dry pyridine. The mixture was left at room temperature for 3 h and was then heated in the water bath with a calcium chloride tube for 4 h. Yield 30 mg. The product was shown to be identical with pachycarpine by its R_f values in three systems.

SUMMARY

The plant Thermopsis lanceolata is richest in cytisine and pachycarpine at the beginning of the vegetation period, which makes it possible to obtain these substances from a single raw material simultaneously. Thermopsamine has been shown to be the optical antipode of the 13-hydroxysparteine obtained by the reduction of hydroxylupanine.

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