REFINEMENT OF THE STRUCTURE OF ASPERUMINE

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Echimidine N-oxide has been obtained previously from Symphytum caucasicum, and the presence of lasiocarpine, echinatinine, and asperumine has been shown chromatographically [1]. We have investigated the roots of this plant collected on October 15, 1971, in the Tashkent botanical garden. The mixture of alkaloids was separated on a column of alumina, and the individual base (I) so obtained was characterized by its crystalline picrate. UV spectrum: λ_{max} 222 nm (log ε 3.9). The IR spectrum has absorption bands (cm^{-1}) 1708, 1730 (ester groups), 1645 (double bonds), and 2800-3500 (active hydrogen). The mass spectrum of (I) has the weak peak of the molecular ion with m/e 397 and strong peaks with m/e 136, 120, 119, 93, and 80, which are characteristic for diesters of pyrrolizidine alkaloids [2].

The picrates of the base (I) and of asperumine [3], and also the N-oxides of these bases, gave no depressions in mixed-melting point tests. Consequently, (I) and asperumine are identical. However, the large difference in their molecular weights and IR spectra has induced us to reconsider the structure of asperumine.

Two isomeric pyrrolizidine alkaloids with mol. wt. 397 are known: heliosupine (II) [4] and echimidine (III) [5]. The results of a direct comparison of the N-oxides of asperumine and of (III), and also the picrates of (I) and (II), showed that asperumine differs from these two isomers.

In the products of the hydrolysis of asperumine, in addition to the heliotridine and angelic acid isolated previously [3], we detected acetone. The same substances are obtained in the hydrolysis of heliosupine [4].

The NMR spectrum of (I) shows a three-proton singlet at 1.77 ppm, a three-proton doublet at 1.92 ppm (J = 7 Hz), and a one-proton multiplet at 6.02 ppm, which are characteristic for angelic acid esters [6], and a nine-proton multiplet in the 1-1.5-ppm region for three $OH-\overset{|}{\underset{|}{C}}-CH_3$ groups. On the basis of the hydrolysis products isolated and the molecular weight and NMR spectrum of asperumine, the latter must be isomeric with echimidine and heliosupine.

Thus, one esterifying acid in asperumine is angelic, and the other must be 2,3,4-trihydroxy-2-methylpentane-3-carboxylic ($C_7H_{14}O_5$).

The difference between (I) and (II) may be which acid esterifies the primary and which the secondary alcohol group, or it may be the stereoisomerism of the $C_7H_{14}O_5$ acid. In the mass spectrum of asperumine the strongest peak is that of the ion with m/e 220 formed by the detachment of the C_7-acid residue. The same ion is the strongest in the mass spectra of (II) and (III) [2].

Consequently, the difference between (I) and (II) is due only to the stereoisomerism of the 2,3,4-trihydroxy-2-methylpentane-3-carboxylic acid.

The hydrogenolysis of asperumine gave asperuminic acid, which must be stereoisomeric with echimidinic acid [5]. The results of a comparison of the properties of these acids are given below.

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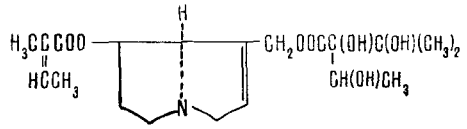
Acid	$[\alpha]_D$, deg (c 1.5, ethanol)	mp of the brucine salt, °C	mp of the quinine salt, °C
Asperuminic	14.8	202—203	188
Echimidinic	16.4 17.5	209—211	—

On the basis of the facts presented, asperumine has the following structure:



EXPERIMENTAL

Isolation of the Total Alkaloids. The finely comminuted dry roots (10 kg) were extracted with 2% H_2SO_4, the acid was concentrated to 4%, and zinc dust was added. After 12 h, the acid solution was filtered, made alkaline, and treated with chloroform. The solvent was evaporated off to small volume, and the alkaloids were extracted with 10% H_2SO_4 and then, after the acid extract had been made alkaline, with methylene chloride and chloroform. These yielded, respectively, 60 g and 3.04 g of mixed bases, amounting to 0.63%.

Preparation of Asperumine. The methylene chloride fraction (3 g) was separated on a column containing 200 g of Al_2O_3. Elution was performed with chloroform and with chloroform–methanol. The fractions showing a single spot on a plate [nonfixed alumina, benzene–ether–methanol (10 : 5 : 2)] were combined. After elimination of the solvents, the asperumine remained in the form of a viscous light-yellow oil.

Asperumine picrate was obtained by mixing ethanolic solutions of the base and the acid, evaporating the solvent, and recrystallizing the residue from a mixture of water and ethanol; mp 130-131°C.

Asperumine N-Oxide. A mixture of 1.27 g of the base and 10 ml of 6% hydrogen peroxide solution was allowed to stand for three days, and then the water was eliminated, the residue was dissolved in methanol, and the N-oxide formed was precipitated with acetone. Yield 1.1 g, mp 152-153°C (decomp.), $[\alpha]_D$ ±0°C; picrate mp 144-145°C (water).

Reduction of the N-oxide with zinc dust in hydrochloric acid yielded aperumine.

Hydrolysis of Asperumine. A mixture of 0.7 g of the base and 10 ml of 2 N aqueous alkali was heated for 2 h. The acetone vapor evolved was trapped in an ethanolic solution of 2,4-dinitrophenylhydrazine. The crystals with mp 115-118°C that deposited gave no depression in admixture with acetone 2,4-dinitrophenylhydrazone.

After acidification with 10% H_2SO_4, the reaction mixture was treated with benzene. The residue from the elimination of the solvent (0.05 g) was crystallized from water, mp 40-40.5°C, which corresponds to angelic acid. The aqueous acid solution was evaporated in vacuum to 8 ml, 2 g of NaOH was added, and the hydrolysis product was extracted with chloroform. The residue consisted of 0.13 g of an oil giving a picrate with mp 117.5-118°C which showed no depression of the melting point with heliotridine picrate.

Hydrogenolysis of Asperumine. A mixture of 2.26 g of the substance and 30 ml of 3.7% hydrochloric acid was shaken in an atmosphere of hydrogen with 0.354 g of platinum oxide for 6 h. After the platinum had been filtered off, the acid solution was made alkaline, and the reaction product was extracted with chloroform. An oily mixture of four substances was isolated which we were unable to separate.

The residual aqueous alkaline solution was acidified and evaporated to dryness, and the residue was repeatedly treated with hot chloroform. This gave 0.5 g of asperuminic acid in the form of a colorless vitreous mass, $[\alpha]_D$ +14.8° (ethanol), brucine salt mp 202-203°C (ethanol), quinine salt mp 188°C (acetone).

SUMMARY

1. Asperumine has been isolated from the combined alkaloids of Symphytum caucasicum.

2. The hydrolysis and hydrogenolysis of asperumine has given heliotridine, acetone, and angelic and asperuminic acids.

3. The structure of asperumine has been refined on the basis of the products of hydrolysis and hydrogenolysis and also of features of the mass and NMR spectra.

4. It has been established that asperumine is an isomer of heliosupine in the 2,3,4-trihydroxy-2-methylpentane-3-carboxylic acid moiety.

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