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ALKALOIDS OF Buxus colchica

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Plants of the genus <u>Buxus</u> (family Buxaceae) are known as containing steroid alkaloids [1-3]. <u>Buxus colchica</u> Pojark (Colchian box) (endemic to the Caucasus) has long been used in folk medicine [3].

Our investigations of this species have shown that the plant is rich in pharmacologically active steroid alkaloids. The total level of bases in the flowering phase in the leaves and green branches found by extraction with ethanol amounted to 3%; and, in the fruit-bearing phase, in the leaves to 0.9%, in green shoots to 0.75%, in lignified branches to 1.1% in the capsules of the fruit to 0.56%, and in the seeds to 1.5%. For the purpose of isolating the total alkaloids and individual bases, we used young branches 15-20 cm long collected in the flowering phase.

The comminuted air-dry raw material was extracted with 80% ethanol. After appropriate purification, the alkaloids were separated into ether-extracted (2.2\%) and chloroform-extracted (0.8\%) fractions.

The ethereal fraction yielded four bases the identification of which was made from a comparison of their physicochemical constants and spectral characteristics (IR, UV, PMR, ¹³C-NMR, and mass spectroscopy) with information in the literature.

Base (I), with the composition $C_{25}G_{42}N_2O$, mp 229-231°C, $[\alpha]_D^{20}$ +90° (c 0.2; chloroform) was isolated when the ethereal fraction was precipitated with a mixture of ethanol and ethyl ether. Compound (I) was identified as pseudocyclobuxine D [4].

After the separation of the pseudocyclobuxine D, the mother liquor was evaporated and the residue was dissolved in ethyl ether and subjected to citrate-phosphate polybuffer separation. Fractions with pH 7, 6, 5, and 4 were obtained. Alkaloids (II) and (III) were isolated from the pH 7 fraction by the methods of reprecipitation and stepwise crystallization.

Base (II), $C_{25}H_{42}N_2O$, mp 236-238°C, $[\alpha]_D^{20}$ +94° (c 0.2; chloroform) proved to be cyclobuxine D [4].

Base (III) with mp 167-170°C $[\alpha]_D^{20}$ +104° (c 0.2; chloroform). Mass spectrum: M⁺ 401. The identification of this alkaloid is proceeding.

Base (IV), $C_{27}H_{48}N_2$, was obtained from the pH 6 fraction by reprecipitation with a mixture of hexane and ethyl ether. After recrystallization, mp 187-189°C $[\alpha]_D^{20}$ -66° (c 0.2; chloroform). Base (IV) was identified as L-cycloprotobuxine C, isolated previously from <u>Buxus sempervirens</u> L. [1, 5].

The investigation of the alkaloids of Buxus colchica is continuing.

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AMINO ACID COMPOSITIONS OF LAVENDER, ROCK ROSE, AND ROSEMARY

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In the industrial processing of essential-oil crops, multitonnage wastes (extracted meal) accumulated which, hitherto, in spite of the known value of the native plants in folk and scientific medicine, have not found due use as additional materials sources for obtaining drugs.

In industry, the essential oil of lavender and of rosemary are obtained by the method of steam distillation, and that of rockrose by extraction with alcohol (resinoid) [3]. We established beforehand that the substances obtained from the extracted meals of these plants are characterized by high antimicrobial, antioxidant, and antiinflammatory, activities. In view of this, it appeared of interest to study the amino acid fraction, and the results of this study are given in the present communication.

To obtain the water-soluble substances, extraction was carried out in hot water at a temperature of 80-100°C and with 40% ethanol at a ratio of raw material to extractant of 1:10. The organic solvent was distilled off from the ethanolic extracts. The elimination of highmolecular-mass compounds was achieved by precipitation with methanol followed by centrifugation (4000 rpm for 20 min). All the extracts were subjected to sublimation drying [1, 2, 5] in the automatic regime using the apparatuses A 650/40/50 (Czechoslovakia) (freezer) and TU 50.4 (GDR) - evaporator. The choice of solvents was based on the solubility of the amino acids, which, as is known, are extracted best by water and aqueous ethanol [4].

To characterize the qualitative composition of the amino acids we used the method of chromatographic analysis, which permitted the revelation of the presence of 14 free amino acids (Leningradskaya S paper, the BAW (4:1:2) system, with a 0.3% solution of ninhydrin as the revealing agent) [5, 6].

The quantitative level of amino acids in the samples investigated was determined with the aid of an AAA 339 amino analyzer (Czechoslovakia) by generally adopted procedures [4, 5]. The calculation and interpretation of the chromatograms was carried out on the associated computer, which was fitted with an analyzer. A solution of 12 mg of each sublimate in 2.2. ml of sodium citrate buffer was introduced into the column of the analyzer. The amount of each identified amino acid was determined in nanograms in an aliquot, and the amount of free aminoacids was then recalculated to mg % on the absolutely dry raw material. The results are given in Table 1.

The analysis showed that the qualitative and quantitative compositions of the samples investigated changed according to the nature of the solvent. The maximum number of amino acids in the lavender and rock rose extracts was 16, and in the rosemary extract 15 (methionine was absent). The fractions of one and the same material (native plant and meal) sometimes differed by 1-2 amino acids. For example, aqueous extracts from lavender (meal and native plant) contained no serine, but it was present in an ethanolic fraction. Aqueous and alcoholic extracts from rockrose meal contained glutamic acid but it was not detected in an aqueous extract from the plant. The amounts of free amino acids in aqueous and alcoholic extracts from rosemary were, respectively, 167.24 and 72.84 mg %; and 286.72 and 191.45 mg %, while in analogous extracts from rock rose they were 512.91 and 552.62 mg %. The high amino acid content in the native rock rose raw material must be mentioned - 1042.29 mg%.

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