LITERATURE CITED

1. S. Kh. Maekh, S. Yu. Yunusov, É. V. Boiko, and V. M. Starchenko, Khim. Prir. Soedin., 227 (1982).
2. S. Kh. Maekh, S. Yu. Yunusov, É. V. Boiko, and V. M. Starchenko, Khim. Prir. Soedin., 791 (1982).
3. H. Achenbach, C. Renner, J. Worth, and J. Addae-Mensah, Liebigs Ann. Chem., 1982, 1132 (1982).

ALKALOIDS OF *Buxus sempervirens*

B. U. Khodzhaev, S. M. Sharafutdinova, A. Dzhabbarov, and S. Yu. Yunusov

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The alkaloids of *Buxus sempervirens* L. (common box) cultivated in the Karachaevo-Cherkeskii autonomous province of Stavropol' krai have not previously been studied.

We determined the alkaloid contents of the various organs of this plant by the chloroform method:

Date of collection (1980)	Plant organ	Total alkaloids, %
May 7	First-year shoots	2.46
	Young roots	2.38
	Leaves and twigs	2.27
	Flowers	2.21
	Roots	2.18
	Perennial twigs	1.02
July 25	First-year shoots	2.34
	Young roots	2.32
	Leaves and twigs	2.01
	Fruit	1.98
	Roots	1.89
	Perennial flowers	0.96

The ethereal fraction of the combined alkaloids isolated from 1.8 kg of twigs and leaves (collected on May 7, 1980) was dissolved in chloroform and separated according to basicities by citrate–phosphate buffer solutions at pH 8.0-2.0 (with a pH interval of 0.5). The fractions of the combined alkaloids with pH 8.0, 7.5, 7.0, and 6.5 were chromatographed separately on a column of alumina (Brockmann activity grade II). Elution was carried out with mixtures of ether and ethanol with increasing concentrations of the latter – 15, 20, 30, 35, and 40%. The fractions indicated yielded cyclobuxine-D, cyclovirobuxine-D, and cycloprotobuxine-A [1-3].

The mother liquor from the cycloprotobuxine-A and the cyclovirobuxine-D was chromatographed on a column of silica gel with elution by hexane–chloroform–ammonia (10:7:0.12). Fractions 25-35 were rechromatographed on a column of alumina with elution by ether–ethanol (5:3) and (5:4).

Fractions 16-23 were treated with acetone. From the acetone-insoluble fraction we isolated a base with mp 233-235°C (ethanol), $C_{26}H_{44}N_2O$ $[\alpha]_D$ +113.24° (c 0.671; chloroform); M^+ 400. This alkaloid was identified as cyclobuxine-B (from a mixed melting point and also the IR, NMR, and mass spectra of the base and of its derivatives) [4].

The combined alkaloids from the pH 6.0 fraction were treated with acetone. From the acetone-insoluble fraction we isolated a base with mp 245-247°C (petroleum ether). The IR spectrum of the alkaloid contained, in addition to others, the absorption bands of activated hydrogen (3315 cm^{-1}), of a methylcyclopropane ring (30364* and 1458 cm^{-1}), and the mass spec-

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trum showed the peaks of ions with m/z 58 (100%), 365, 383, 407, 424, and M^+ 440, characteristic for the mass-spectrometric fragmentation of cycloprotobuxine bases [5]. From its spectral characteristics, this alkaloid belongs to bases of the 9β,19-cyclo-5α-pregnane type, which is different from the alkaloids of the genus *Buxus* isolated previously.

Thus, from *Buxus sempervirens* L. from a growth site not studied previously we have isolated cyclobuxine-D, cyclovirobuxine-D, cycloprotobuxine-A, cycloprotobuxine-B, and a base with mp 245-247°C.

LITERATURE CITED

1. B. U. Khodzhaev, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 542 (1971).
2. B. U. Khodzhaev, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 114 (1974).
3. S. M. Kupchan and E. Kurosawa, J. Org. Chem., 30, No. 6, 2046 (1965).
4. Z. Voticky, V. Paulik, and B. Sedlak, Chem. Zvesti, 23, 702 (1969).
5. I. Tomko, Z. Voticky, V. Paulik, A. Vassova, and O. Bauerova, Chem. Zvesti, 18, 721 (1964).

CYANOGEN BROMIDE HYDROLYSIS OF RAT TRANSFERRIN

A. A. Buglanov, T. A. Salikhov, and B. A. Tashpulatova

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The transferrins form a group of homologous glycoproteins capable of reversibly binding to ferric iron ions in two centers present in the protein molecule [1]. The results of investigations on the structure of protein homologs of the transferrin type give grounds for assuming that the transferrin molecules with two centers for the reversible binding of iron ions have as their ancestor a protein molecule with one center for binding iron ions and that this phenomenon is the result of a doubling of the gene of the transferrin precursors taking place in the course of evolution.

We have made an attempt to obtain cyanogen bromide fragments of rat transferrin obtained by a method described previously [2] with the aim of their subsequent separation and study.

According to the results of analysis of amino acid composition of native transferrin, showing the presence of methionine residues in the protein, one may theoretically expect the production of five fragments after hydrolysis with cyanogen bromide. Hydrolysis with cyanogen bromide was carried out in the following way: 70 mg (1 μmole) of the protein was dissolved in 3 ml of 70% formic acid, and 3 ml of a solution of cyanogen bromide (1 g of cyanogen bromide/10 ml of 70% formic acid) containing a 100-fold excess of cyanogen bromide with respect to the methionine was added. The reaction was carried out for 48 h in an atmosphere of nitrogen in the dark at +4°C with gentle stirring. The reaction mixture was evaporated in a rotary evaporator to a volume of ~3 ml. An aliquot of the reaction mixture was subject to electrophoresis in polyacrylamide gel (PAAG) by Weber and Osborn's method [3]. The electrophoretogram showed the presence of five bands corresponding to five cyanogen bromide fragments.

The fragments obtained after hydrolysis were separated on a column of Sephadex G-50 equilibrated with 35% acetic acid at a rate of flow of 15 ml/h. Fractions with a volume of 5 ml were collected and the peptide material in an eluate was recorded from its absorption at 280 nm on a Gilson spectrophotometer (France). The results of separation are shown in Fig. 1. The peptide fractions present in the peaks were evaporated in a rotary evaporator to a volume of ~1 ml. The chromatographic peaks were denoted, respectively, BF-1, BF-2, BF-3, BF-4, and BF-5.

Each peptide fragment was checked for homogeneity by electrophoresis in PAAG in the presence of 1% of sodium dodecyl sulfate. The pattern of electrophoresis of aliquots from the fractions obtained on gel filtration indicated substantial aggregation in fractions BF-1,

Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent.

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