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ALKALOIDS OF *Buxus colchica*, *B. sempervirens*, and *B. balearica* GROWING IN GEORGIA

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The introduced species *Buxus sempervirens* and *B. balearica* grow in Georgia in addition to the endemic *B. colchica* Pojark.

We studied leaves and stems of these plants collected during flowering and fruiting near Tbilisi, in Batum Botanical Garden, and on Shaor Pass.

Pharmacological tests of total preparations that were performed at the Kutateladze Institute of Pharmaceutical Chemistry showed that total alkaloids soluble in ether, $CHCl_3$, and water exhibited spasmolytic activity. The strength of the activity of the aqueous extracts of the *Buxus* species could be placed in the following order [1]: *B. balearica* Lam > *B. sempervirens* > *B. colchica* Pojark.

Plant material was extracted beforehand with EtOAc followed by extraction of alkaloids by diethylether and $CHCl_3$ after making the raw material basic in order to obtain total alkaloids. Total alkaloids soluble in water were obtained as an extract from leaves and stems of *B. colchica*, *B. sempervirens*, and *B. balearica* according to requirements of the SP, XIth Ed. (1982, Vol. II, p. 160).

Seasonal variations of alkaloids in vegetative organs were studied by obtaining total alkaloids in a Soxhlet apparatus using extraction by $CHCl_3$ and basic raw material with subsequent acid–base purification and separation into ether and $CHCl_3$ fractions.

Total alkaloids soluble in ether were separated using citrate–phosphate buffers in pH steps 6.6, 6.0, 5.0, 4.0, and 3.0 with 0.1 M citric acid. Separation into pure bases was carried out over Al_2O_3 (activity II, III, VI, and neutral) and SiO_2 (100/160 μm) columns. The ratio of compounds to sorbent was 1:100. Elution used ether, benzene:ether (1%), benzene:EtOH (1–5%), benzene:$CHCl_3$, $CHCl_3$:CH_3OH with increasing MeOH content. Fractions of 25–50 mL were collected.

The yield of total alkaloids was determined by acid–base titration calculated as molecular weight of buxamine; the buxamine content, by a chromatographic titration method using *B. colchica* and *B. sempervirens*. Qualitative analysis was performed in thin layers of SiO_2 (Merck) on plates using MeOH:hexane:NH_4OH (25%) (40:5:3). The yield of total alkaloids from *B. balearica* was determined gravimetrically [2].

Alkaloids were identified based on a determination and analysis of physicochemical (mp, $[\alpha]_D$) and spectral data (UV, IR, PMR, mass) in addition to comparison with the literature [1-9]. Reference samples for TLC mobility were authentic alkaloids. Table 1 shows the variations in alkaloid contents with development phases in the vegetative organs.

B. balearica, yield of total alkaloids from leaves and stems during flowering, 6%. The fraction from pH 0.6; 6.5 of the ether total was separated over an Al_2O_3 column with elution by benzene:$CHCl_3$ and benzene:EtOH (1→5%). Fractions from EtOH (72–126) afforded **1**, mp 199–201°C (EtOH), $[\alpha]_D$ –100.3° ($CHCl_3$).

Mass spectrum (*m*/*z*): 414 $[M]^+$, 386, 371, 356, 343, 340, 84, 72 (100), 58. PMR spectrum ($CDCl_3$, δ, ppm): 0.74 (3H, s, CH_3), 0.88 (6H, s, 2 × CH_3), 0.92 (6H, s, 2 × CH_3), 2.2 [6H, s, $N(CH_3)_2$], 2.40 [6H, s, $N(CH_3)_2$].

The experimental results allowed us to identify the isolated compound as cycloprotobuxin-A [4, 5].

B. sempervirens, yield of total alkaloids, 2.2%. Amorphous base **2** was isolated from the fraction with pH 5.0 of the ether total alkaloids by separation over a column of Al_2O_3 with elution by benzene:ether (1%) from effluents (9–12).

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TABLE 1. Alkaloid Content in Vegetative Organs in *Buxus* Species by Vegetation Phase

Collection site	Plant organ	Content in ether of soluble total, % calc. per air-dried raw matl.		Content of buxamine, % calc. per air-dried raw matl.	
		flowering phase	fruiting phase	flowering phase	fruiting phase
		Buxus colchica Pojark.			
Shaor Pass	1st-year runners	1.25	1.75	0.062	0.065
	Leaves and stems	1.55	1.88	0.076	0.082
	Flowers	1.00	–	0.04	–
	Fruit	–	1.80	–	0.080
	Yellowed leaves and thin branches	–	1.15	–	0.060
	Aged branches	0.92	0.94	0.096	0.090
Black Sea shore	Leaves and stems	1.45	1.88	0.063	0.074
		Buxus balearica Lam			
near Tbilisi	1st-year runners	2.08	2.62	–	–
	Leaves and stems	2.3	2.0	–	–
		Buxus sempervirens			
near Tbilisi	1st-year runners	1.12	1.38	0.01	–
	Leaves and stems	1.08	0.92	0.011	–

Compound **2**, $[\alpha]_D$ +34 (*c* 0.4, $CHCl_3$). UV spectrum (CH_3OH, λ_{max}, nm): 239, 247, 256, inflection near 290. IR spectrum (cm^{-1}): 1600, 1585, 1372, 1360, 1325, 1250, 1070, 1021, 982, 880, 830. PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69, 0.71, 0.74, 0.99 (12H, s, 4 × CH_3), 1.1 (3H, d, J = 6, C^{21}–CH_3), 2.28 [6H, s, $N(CH_3)_2$]. Mass spectrum (*m*/*z*): 384 (5) $[M]^+$, 84 (33), 71 (70), 58 (30), 44 (100).

A comparison of the results with the literature identified the compound isolated by us as buxamine [4, 6, 7, 9].

The alkaloid cycloprotobuxin-A was isolated and identified from the fraction with pH 6.5 analogously to the separation of the *B. balearica* alkaloids.

B. colchica, column separation over Al_2O_3 of ether total buffer fractions isolated and identified cyclobuxin-D, pseudocyclobuxin-D, L-cycloprotobuxin-C, cycloprotobuxin-D, 3-*N*-dimethyl-C_{20}N-methylaminocycloprotobuxin-C, and pseudocyclobuxin-D (-)-*N*-oxide [1].

The alkaloids buxamine and cycloprotobuxin-A were isolated and identified by column separation of the ether soluble total from fractions with pH 0.6; 5.0 analogously as for *B. balearica* and *B. sempervirens*.

The $CHCl_3$ total (45 g) was separated over a column of SiO_2 with elution by $CHCl_3$ with increasing MeOH content. Fractions with $CHCl_3$:CH_3OH (20:80) afforded an amorphous base. UV spectrum (λ_{max}, nm): 238, 247, 253. IR spectrum (ν_{max}, $CHCl_3$, cm^{-1}): 1375 (C–N), 3450-3550 (–OH). Mass spectrum (*m*/*z*): 444 (4), 428 (13), 429 (13), 414 (13), 398 (8), 381 (15), 367 (15), 354 (15), 198 (45), 181 (15), 154 (17), 129 (5), 84 (11), 72 (100), 71 (36), 58 (32).

Comparison of the results with the literature identified the isolated base as buxaminol-G [8].

Buxamine and buxaminol-G were found for the first time in *B. colchica.*

Table 1 shows that alkaloids accumulate most in leaves and stems of *B. colchica* during fruiting (1.88%); buxamine, during flowering (0.096%). Comparison of the qualitative and quantitative compositions of the total alkaloids soluble in ether that were obtained from plants growing on the Black Sea shore and in Shaor Pass showed that the spectra of alkaloids in plant specimens from both regions that were collected during fruiting were practically identical. Therefore, plants growing in these sites can be used equally for preparing pharmacologically active alkaloids. Leaves and stems of *B. colchica* should be collected during seed ripening in order to obtain buxamine. *B. sempervirens* produced less buxamine than *B. colchica*. With respect to *B. balearica*, this plant produced large quantities of alkaloids and, as noted above, was superior to *B. sempervirens* and *B. colchica* with respect to pharmacological activity.

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