Buxus alkaloids. XIV.*
Alkaloids from *Buxus sempervirens* var. *angustifolia* WEST.

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Ten alkaloids were identified by spectral methods and comparison with authentic specimens in the extract of leaves from *Buxus sempervirens* var. *angustifolia* WEST.: buxamine-E, buxaminol-E, buxenine-G, buxpiine-K, buxtauine-M, cyclobullatine-A, cyclobuxamine-H, cyclobuxine-D, cycloprotobuxine-C, and cyclovirobuxine-D. The main alkaloid of this plant was found to be cyclobuxamine-H.

В экстракте листьев *Buxus sempervirens* var. *angustifolia* WEST. мы установили спектральными методами и сравнением с подлинными пробами десять алкалоидов: буксамин-E, буксаминол-E, буксенин-G, букспиин-K, букстауин-M, циклобулатин-A, циклобуксамин-H, циклобуксин-D, циклопротобуксин-C и цикловиробуксин-D. Основным алкалоидом этого растения является циклобуксамин-H.

Various species of common box *(Buxus)* are cultivated in the Arboretum of the Slovak Academy of Sciences in Mlyňany under the same soil and climatic conditions; consequently, the examination of alkaloids produced by those plants is of chemotaxonomical importance. From this locality we investigated so far alkaloids from *Buxus sempervirens* L.[1, 2], *B. microphylla* SIEB. et ZUCC. var. *sinica* REHD. et WILS. [3], *B. sempervirens* var. *bullata* KIRCHN. [4], *B. sempervirens* var. *argentea* HORT. ex STEUD. [5], and *B. sempervirens* var. *angustifolia* WEST. Alkaloids present in the last species were found also in other plants of the genus *Buxaceae* with the difference that the main alkaloid of *Buxus sempervirens* var. *angustifolia* was cyclobuxamine-H. In addition to alkaloids the presence of sterols and triterpenes in *Buxus sempervirens* L. was investigated [6]; these substances could be intermediates in the biosynthesis of alkaloids.

Experimental

Melting points were determined on a Kofler micro hot-stage, the optical rotation of chloroform solutions was measured with a Perkin—Elmer 141 apparatus in 1 cm cells. Mass spectra were taken with a MCh 1306 spectrometer (USSR) adapted for a direct introduction of the sample to the ionization chamber at ionizing electron energy 70 eV and 1 mA intensity. Infrared spectra were recorded with a Perkin—Elmer 457 spectrometer in KBr, ultraviolet spectra with an ORD/UV-5 Jasco apparatus in ethanol. Alumina Merck (activity grade II) was used for column chromatography; purity of alkaloids

* For Part XIII see Ref. [5].

was monitored by t.l.c. chromatography, alumina Reanal (activity grade VI) being the support and benzene—chloroform—ethanol 8 : 12 : 0.3—5.0 (according to the polarity of substances investigated) the solvent system.

Isolation of alkaloids

The plant material (terminal twigs of *Buxus sempervirens* var. *angustifolia* WEST 6.5 kg) was collected at the end of September 1972. The woody parts being removed, the dry, ground leaves were macerated 5 times with acetic acid acidified (5%) dilute methanol (50%, 175 l total). The organic solvent was removed under diminished pressure and the acid aqueous solution of bases was worked up as described earlier [3]. The mixture of alkaloids thus obtained (118 g, 1.8%) was dissolved in chloroform to give 5% solution, which was extracted stepwise with the respective McIlvain buffer solutions (pH 6.5, 6.0, 5.0, 4.0, 3.0) and finally with 2% hydrochloric acid. Bases liberated by ammonia from the individual pH portions were extracted with chloroform and separated by column chromatography. The distribution of alkaloids in the particular pH portions is shown in Table 1.

Table 1

Distribution of alkaloids in the individual pH portions

Portion pH	%	Alkaloid	mg	Ref.
6.6	11.9	Cyclobuxine-D	64.2	[1, 2]
		Cyclovirobuxine-D	51.4	[7]
		Buxenine-G	71.7	[8, 9]
		Cyclobuxamine-H	2379.5	[10]
6.0	13.1	Cyclobuxine-D	4.5	
		Cyclovirobuxine-D	186.9	
		Cyclobuxamine-H	27.0	
		Buxaminol-E	14.4	[8]
		Cyclobullatine-A	13.1	[4]
5.0	11.1	Buxaminol-E	13.1	
		Cycloprotobuxine-C	1020.8	[11]
		Buxamine-E	47.0	[8]
		Buxtauine-M	9.2	[12]
4.0	10.7	Buxtauine-M	557.0	
3.0	5.3	Buxpiine-K	34.5	[12]
2% HCl	1.9	—		
Residue in $CHCl_3$	37.2	—		
Loss	8.8	—		

Identification and characterization of alkaloids

Cyclobuxine-D: m.p. 243°C (acetone—methylene chloride), $[\alpha]_D^{23}$ +97° (*c* 1.2). The mass spectrum of this substance revealed a peak of the molecular radical ion at *m/e* 386 and a fragmentation pattern *m/e* M—15, M—30, M—58 and 44, 57, 58, 70 diagnostic of a methylamino substitution at C-20 and C-3, respectively. The intensity of peaks at *m/e* 57 and 70 was markedly lowered due to the presence of an exocyclic methylene group at C-4 in the neighbourhood of one methylamino group [13]. The infrared spectrum was identical with that of an authentic specimen.

Cyclovirobuxine-D: m.p. 221°C (acetone—methylene chloride), $[\alpha]_D^{23}$ +63° (*c* 1.0). This base was recognized by a typical fragmentation pattern of methylamino groups at C-3 and C-20, by the peak of molecular radical ion at *m/e* 402, further by absorption bands in the infrared spectrum ascribable to vibrations of a hydroxyl (1035 and 3305 cm^{-1}), a *gem*-dimethyl (1376 cm^{-1}), and a cyclopropylmethylene (1460 cm^{-1}) groups and by a mixed melting point with the authentic specimen.

Buxenine-G: amorphous, $[\alpha]_D^{20}$ +20° (*c* 0.9). *N'*-Isopropylidenebuxenine-G: m.p. 190°C (acetone), $[\alpha]_D^{20}$ +38° (*c* 0.8). The mass spectrum of buxenine-G showed, in addition to the molecular radical ion at *m/e* 370, peaks indicative of a methylamino fragmentation pattern at C-3 and an amino fragmentation pattern at C-20. The absorption maxima in the ultraviolet region at 238, 247, and 255 nm (log ε 4.4, 4.5, and 4.2) were associated with a conjugated heteroannular diene; absorption bands of the respective *gem*-dimethyl group, amino group, and a diene grouping in the infrared spectrum (1378, 1578, and 1630 cm^{-1}) were concordant with those reported in [9].

Cyclobuxamine-H: amorphous, $[\alpha]_D^{22}$ +28° (*c* 1.0). *N*-Isopropylidenecyclobuxamine-H: m.p. 244°C (acetone, decomposition), $[\alpha]_D^{25}$ +66° (*c* 1.3). The identity of this alkaloid was verified by means of its mass spectrum displaying a peak of the molecular radical ion at *m/e* 374 and a fragmentation characteristic of a methylamino substitution at C-20 and an amino substitution at C-3, by its infrared spectrum with bands corresponding to vibrations of a hydroxyl, a cyclopropylmethylene, and a methylamino groups (1053, 1458, 3320 cm^{-1}, respectively) and also by the accordance of its physicochemical constants with the published data [10].

Buxaminol-E: amorphous, $[\alpha]_D^{22}$ +36° (*c* 0.5). *N'*-Isopropylidene derivative: m.p. 206—207°C (acetone), $[\alpha]_D^{22}$ +95° (*c* 0.6). This base displayed an identical ultraviolet spectrum with buxenine-G; infrared spectra of both substances differed in the 1035 cm^{-1} region only (hydroxyl group). The mixed melting point of *N'*-isopropylidenebuxaminol-E with an authentic specimen did not show any depression.

Cyclobullatine-A: m.p. 275°C (acetone), $[\alpha]_D^{22}$ −99° (*c* 0.6, ethanol). The mass spectrum revealed, in addition to the peak of the molecular radical ion at *m/e* 414, a fragmentation characteristic of dimethylamino substitutions at C-3 and C-20. The absorption in the ultraviolet region at 204 nm (log ε 3.63) indicated the presence of an isolated double bond. The bands in the infrared region were associated with the vibrations of a hydroxyl (1095 cm^{-1}), *tert*-amino (1260, 2760, 2778, 2815 cm^{-1}) cyclopropylmethylene (1415, 3030 cm^{-1}), and *sec*-methyl (1380, 2958 cm^{-1}) groups. All these data were in agreement with the corresponding data of the authentic specimen.

Cycloprotobuxine-C: m.p. 210°C (acetone), $[\alpha]_D^{22}$ +69° (*c* 0.6). According to the mass spectrum this alkaloid was a two-nitrogen containing base of molecular weight 400, having a dimethylamino group at C-20 and a methylamino substitution at C-3. The infrared spectrum was superimposable with that of the authentic specimen.

Buxamine-E: amorphous, $[\alpha]_D^{22}$ +32° (*c* 0.8). *N'*-Isopropylidenebuxamine-E: m.p. 177°C (acetone), $[\alpha]_D^{22}$ +44° (*c* 0.17). Buxamine-E is the *N*-methyl derivative of buxenine-G and their ultraviolet spectra are identical. The infrared spectra of both alkaloids differed in the region of N—H stretching vibrations only. The mass spectrum evidenced the molecular weight 384, a dimethylamino substitution at C-3 and a free amino group at C-20.

Buxtauine-M: m.p. 178°C (acetone), $[\alpha]_D^{23}$ +154° (*c* 0.9). The base peak in the mass spectrum appeared at *m/e* 43 (acetyl grouping) together with peaks of lower abundance indicative of a methylamino group at C-3 adjacent to an exocyclic double bond. Both mass and infrared spectra were identical with those of the authentic specimen.

Buxpiine-K: m.p. 173°C (acetone), $[\alpha]_D^{21}$ +158° (*c* 1.0); it is the *N*-methyl derivative of buxtauine-M. The different substitution at C-3 was indicated by the mass spectrum. The identity of this alkaloid was confirmed by comparison of both mass and infrared spectra with those of the authentic specimen and by no depression showing mixed melting point.

Spectra of all isolated alkaloids were recorded in the Department of Analytical Chemistry, Institute of Chemistry, Slovak Academy of Sciences (Head C. Peciar).

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