Buxus alkaloids
XXII.* Alkaloids of leaves from immature twigs of *Buxus sempervirens* var. *angustifolia* WEST.

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Seven alkaloids were isolated from the leaves of immature twigs of *Buxus sempervirens* var. *angustifolia* WEST.: cyclovirobuxine-D, buxaminol-E, cyclobuxine-D, buxtauine-M, buxpiine-K, cycloprotobuxine-C, and cyclosuffrobuxine-K, cyclovirobuxine-D being the principal base. Comparison with the results of isolation from mature leaves of the same species showed substantial differences in both kind and quantity in relation with the time of collection of the drug. These results might be of importance when studying the biosynthesis of Buxus alkaloids and their mutual transformations.

Изолировано семь алкалоидов из листьев молодых саженцев *Buxus sempervirens* var. *angustifolia* WEST.: цикловиробуксин-D, буксаминол-E, циклобуксин-D, букстауин-M, букспиин-K, циклопротобуксин-C и циклосуффробуксин-K, причем цикловиробуксин-D является главным основанием. Сравнение с результатами выделения веществ из зрелых листьев тех же растений показало существенные различия как в качественном, так и в количественном составе в зависимости от времени сбора препарата. Эти результаты могут иметь значение при изучении биосинтеза алкалоидов Buxus и их взаимных превращений.

Isolation and identification of alkaloids from the extract of leaves of the mature twigs of *Buxus sempervirens* var. *angustifolia* WEST. have already been reported in one of our preceding communications [2].

Young sprouts grown after a preceding early summer trimming (immature) were collected at the same locality (Botanical garden of the Slovak Academy of Sciences at Mlyňany), at the same time and worked up by the same procedure. The extract of immature leaves showed a considerable difference in both the alkaloid content and representation; also the amount is markedly lower and makes 0.39 % on the

* For Part XXI see Ref. [1].

mass of dry drug. Nevertheless, the extract contains even less nonbasic metabolites and consequently, the content of alkaloids is relatively by twice greater than that of mature drug, where it amounts 1.8 %. Papers dealing with isolation of alkaloids from various species and varieties of the Buxus family from this locality have reported [1—7] the yield of crude bases within 1 to 2.8 %. Differences between the two crops of the same species are listed in Table 1. The per cent representation of cyclovirobuxine-D, the principal alkaloid of immature twigs, is three times higher than that of cyclobuxamine-H, which is the main alkaloid of mature twigs; it could be anticipated that the former, having one more methyl group at the nitrogen attached to C-3, plays certain role in the formation of alkaloids. The next most populated base is buxaminol-E with an extended B-ring of the steroid backbone; further compounds of the same structural type, buxenine-G and buxamine-E, were not identified at all, whereas in mature twigs they were present in low quantities. Cyclobuxine-D, the main alkaloid of *Buxus sempervirens* L., is the third most populated base and in mature twigs its content is by one order of per cent lower. A substantial difference has also been found with cycloprotobuxine-C present in a minute amount in immature, but dominating in mature twigs. Another missing alkaloid of the cyclopropane type was, in addition to the already mentioned cyclobuxamine-H, cyclobullatine-A. The one nitrogen-containing bases buxtauine-M and its *N*-methyl analogue buxpiine-K were found in low quantities, whereas in mature twigs buxtauine-M belongs to the most represented bases. Cyclosuffrobuxine-K, obtained in a little amount was not found in mature twigs.

Table 1
Alkaloids isolated from *Buxus sempervirens* var. *angustifolia* WEST.

Alkaloid	Immature		Mature [2]	
	Yield		Yield	
	m/mg	*w*/mass %	*m*/mg	*w*/mass %
Cyclovirobuxine-D	1713.5	6.64	238.0	0.27
Buxaminol-E	139.7	1.13	27.5	0.02
Cyclobuxine-D	88.0	0.34	68.7	0.05
Buxtauine-M	11.3	0.05	566.2	0.48
Cycloprotobuxine-C	2.1	0.04	1020.8	0.86
Buxpiine-K	5.3	0.02	34.5	0.03
Cyclosuffrobuxine-K	5.2	0.02	—	—
Cyclobuxamine-H	—	—	2406.5	2.04
Buxenine-G	—	—	71.7	0.06
Buxamine-E	—	—	47.0	0.04
Cyclobullatine-A	—	—	13.1	0.01
Total	1965.1	8.24	4494.0	3.80

All the presented differences might be useful for the study of biosynthesis of Buxus alkaloids and their mutual transformations in relation to the various stages of ontogenesis.

Experimental

Melting points were determined on a Kofler hot-stage, the optical rotation of chloroform solutions was measured in a 1 cm cell with a Perkin—Elmer, model 141, apparatus. Electron impact mass spectra were taken at 70 eV and 12 eV ionization energy and 300 μA trap current with a JMS 100D (Jeol) instrument (temperature of the ionization chamber 200 °C, vapourization temperature 180—220 °C). Infrared spectra were recorded with a Perkin—Elmer, model 457, spectrophotometer in KBr discs, ultraviolet spectra of methanolic solutions with a Beckman DB-GT apparatus. For column chromatography alumina Merck (basic, Brockmann II) was used. The purity of alkaloids was monitored on loose-layer plates coated with alumina Reanal (neutral, Brockmann VI) in benzene—chloroform—ethanol (volume ratio = 8 : 12 : 0.5).

Isolation of alkaloids

The drug (young sprouts of *Buxus sempervirens* var. *angustifolia* WEST., 6.7 kg) was collected in September 1978 and worked up as already reported [4]. Yield of the crude extract was 25.8 g (0.39 %). Distribution of the extract into pH fractions and yields of the particular alkaloids are summarized in Table 2.

Table 2

Distribution of the extract into pH fractions and yield of alkaloids isolated

pH	*w*/mass %	Alkaloid	Yield/mg
6.5	33.0	Cyclovirobuxine-D	1713.5
		Cycloprotobuxine-C	2.1
		Cyclobuxine-D	26.0
6.0	9.7	Buxaminol-E	122.0
		Cyclosuffrobuxine-K	5.2
5.0	5.9	Cyclobuxine-D	62.0
		Buxaminol-E	17.7
		Cyclovirobuxine-D	13.5
4.0	3.9	Oil*	31.0
3.0	3.9	Buxtauine-M	11.3
2 % HCl	3.9	Buxpiine-K	5.3
The residue in $CHCl_3$	25.1		
Loss	14.6		

* Mixture of two alkaloids resisting resolution.

Identification and characterization of alkaloids

Cyclovirobuxine-D: m.p. (benzene—ethanol) = 220—221 °C, $[\alpha]_D^{21} = +63°$ (ϱ = = 0.96 %). Mass spectrum, $m/z = 402$ (M^+) and a typical fragmentation of C-3 and C-20 methylamino groups [8]. Infrared spectrum was identical with that of the specimen, mixed melting point without depression.

Buxaminol-E: amorphous, $[\alpha]_D^{23} = +36°$ ($\varrho = 0.8$ %), mass spectrum, $m/z = 400$ (M^+). Absorption bands in the u.v. region, identical with the specimen [9], were indicative of the heteroannular conjugated diene. Stretching vibrations of the i.r. spectrum were diagnostic of the hydroxyl group, double bond, amino and dimethylamino, and methyl groups. *N'*-Isopropylidenebuxaminol-E was obtained from the cooled acetone solution of buxaminol-E after boiling and concentration as crystals; m.p. = 207 °C, $[\alpha]_D^{23} = +95°$ ($\varrho = 0.98$ %).

Cyclobuxine-D: m.p. (benzene—ethanol) = 242 °C, $[\alpha]_D^{23} = +97°$ ($\varrho = 1.0$ %). Mass spectrum, $m/z = 386$ (M^+), and fragmentation pattern in accordance with that reported for this base [10]. Also the i.r. spectra were identical and the mixed melting point was without depression.

Buxtauine-M: m.p. (acetone) = 177—178 °C, $[\alpha]_D^{24} = +156°$ ($\varrho = 0.68$ %). Mass spectrum, $m/z = 371$ (M^+), and a series of ions indicative of C-3 methylamino and C-20 carbonyl substitution; the lowered intensity of peaks of fragment ions at $m/z = 44$, 57 and 70 evidenced the presence of an exomethylene group at C-4 [11]. The mixed melting point with the specimen had no depression [12].

Buxpiine-K: m.p. (acetone) = 173 °C, $[\alpha]_D^{23} = +158°$ ($\varrho = 0.78$ %). Mass and i.r. spectra and the optical rotation value were in line with those of the specimen [12].

Cycloprotobuxine-C: m.p. (acetone) = 209—210 °C, $[\alpha]_D^{21} = +68°$ ($\varrho = 0.82$ %). Mass spectrum was typical of the fragmentation pattern of dimethylamino group at C-20 and a methylamino group at C-3; the peak at $m/z = 400$ met requirement for this base and so did the absorption bands in the i.r. spectrum. The mixed melting point showed no depression with the specimen [13].

Cyclosuffrobuxine-K: m.p. (benzene—ethanol) = 165—167 °C, $[\alpha]_D^{21} = -83°$ (ϱ = = 0.24 %). Mass spectrum, $m/z = 367$ (M^+), and other peaks of ions were indicative of a dimethylamino substitution at C-3. Absorption bands in the u.v. region proved the presence of an α, β-unsaturated carbonyl at the five-membered ring. The presented data, the melting point without depression, and the optical rotation value corroborated the structure assignment [14].

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