Buxus alkaloids. XIII.*
Alkaloids from *Buxus sempervirens* var. *argentea* HORT. ex. STEUD.

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Twelve steroid alkaloids were isolated from *Buxus sempervirens* var. *argentea* HORT. ex. STEUD. Nine of them: buxtauine-M, cyclobuxamine-H, cyclobuxine-D, cyclobuxophyllinine-M, cyclobuxoviridine-L, cycloprotobuxine-C, cycloprotobuxine-D, cyclovirobuxine-D, and irehine were separated also from other Buxus plants. Structural formula of an alkaloid of molecular weight 386, denominated cyclobuxargentine-G, was postulated on the basis of spectral evidence. Two alkaloids of molecular weight 398 and 426 were characterized by physicochemical constants and spectral data.

Из *Buxus sempervirens* var. *argentea* HORT. ex. STEUD. мы изолировали двенадцать стероидных алкалоидов. Девять из них: букстауин-М, циклобуксамин-Н, циклобуксин-D, циклобуксофилинин-М, циклобуксовиридин-L, циклопротобуксин-С, циклопротобуксин-D, цикловиробуксин-D и ирегин нам знакомы. Алкалоиду молекулярной массы 386 мы на основе спектральных данных установили структуру и назвали его циклобуксаргентин-G. Два алкалоида молекулярной массы 398 и 426 мы характеризовали физико-химическими константами и спектральными данными.

Although alkaloids of the *Buxaceae* plants were already isolated in the first half of the 18th century [1], the structure of these substances remained unknown till 1962, when the constitution [2] and configuration [3] of cyclobuxine-D, the principal alkaloid of *Buxus sempervirens* L., were elucidated. Hitherto, alkaloids in several species of the *Buxaceae* family [4] and more recently also sterols and triterpenes in *Buxus sempervirens* L. [5] were examined.

We wish now to report the isolation and identification of nine alkaloids of *Buxus sempervirens* var. *argentea* HORT. ex. STEUD.; the constitution of an alkaloid of molecular weight 386, denominated cyclobuxargentine-G, was postulated basing upon spectral data. The structures of additional two bases of molecular weight 398 and 426 could not be inferred because of the lack of material.

The mixture of alkaloids prepared in a usual procedure from the dry, ground drug [6] was separated by a gradient extraction of the chloroform solution with McIlvain buffer solutions and with dilute hydrochloric acid.

Thus cyclovirobuxine-D [7], cyclobuxine-D [2, 3], cycloprotobuxine-C [8], cycloprotobuxine-D [9], and cyclobuxamine-H [10] were identified in the pH 6.5 portion. From the pH 6.0 portion an additional amount of cycloprotobuxine-C and cycloprotobuxine-D was separated. The main alkaloid of the pH 5.0 fraction was found to be buxtauine-M [11];

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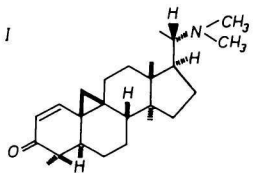
a new base B-426, the mass spectrum of which displayed peaks at *m/e* 426 (M^+), 70, 57, 44, 43, was prepared in a minute amount. The data obtained did not allow to make structural assignments for this alkaloid.

A base having according to the mass spectrum, molecular weight 383 and fragmentation pattern indicative of a dimethylamino substitution at C-20 [*m/e* 368 (M-15), 339 (M-44), 309 (M-72) and 72] [12] was separated from the pH 4.0 portion. The absorption band in the ultraviolet spectrum at 267 nm (log ε 4.0) could be ascribed to an α,β-unsaturated ketone. The infrared spectrum was diagnostic of the presence of a conjugated 6-membered ketone (1602, 1659 cm^{-1}), and a cyclopropane ring (1460, 3045 cm^{-1}). The p.m.r. signals were indicative of following proton resonances (on the δ scale in p.p.m.): 0.76 (1H, one of the AB doublets, $J = 4.5$ Hz) cyclopropylmethylene; 0.95 (3H, s), 0.97 (6H, s), 1.11 (3H, s) four *tert*-methyl groups; 0.86 (3H, d, $J = 6.5$ Hz) *sec*-methyl group; 2.20 (6H, s) dimethylamino grouping; 5.91 and 6.76 (2H, AB doublets, $J = 10$ Hz) two olefinic protons. The experimental data virtually coincided with those reported for cyclobuxoviridine-L (*I*) [13] with the only difference that the alkaloid isolated in our laboratory displayed a negative, nevertheless absolutely the same value of optical rotation. We are unable to rationalize this fact at the time being.



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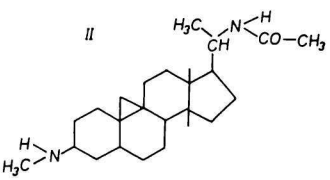

II

Further alkaloid of this fraction, B-398 was isolated in an amount allowing just to record the mass spectrum: the molecular radical ion peak appeared at *m/e* 398 and the fragmentation was typical of a C-3 methylamino (*m/e* 44, 57, 70) and C-20 dimethylamino (*m/e* M-15, M-44, M-57 and 72) substitutions. The last alkaloid of this pH portion was irehine [14]. In the pH 3.0 fraction an additional amount of cyclobuxoviridine-L, cyclobuxophyllinine-M [13] and a new base, to which we propose the name cyclobuxargentine-G, were found. Its mass spectrum revealed peaks at *m/e* 386 (M^+) and 43 ($CH_3—C\equiv O^+$). The series at *m/e* 44, 57, and 70 evidenced the C-3 methylamino group. The infrared spectrum showed bands associated with the vibration of an amide (1527 and 1638 cm^{-1}), cyclopropylmethylene (1450 and 3050 cm^{-1}), and a *sec*-amino group (3330 cm^{-1}). The ultraviolet spectrum was transparent up to 203 nm. The p.m.r. spectrum could not be taken due to the unsufficient amount of the alkaloid under investigation. The obtained data would allow (stereochemistry excluding) to postulate for cyclobuxargentine-G a tentative formula *II*. Cyclobuxargentine-G is, besides of buxandonine-L [15], another buxus alkaloid without a substituent at C-4. This structural type is rarely represented [16] and so is also the acetyl group attached to nitrogen at C-20 [15, 17].

Experimental

Melting points were measured on a Kofler micro hot-stage, optical rotation of chloroform solution with a Perkin—Elmer 141 spectrometer in 1 cm cells. The mass spectra were recorded with a MCh 1306 (USSR) spectrometer adapted for a direct introduction of the sample to the ionization chamber at a ionizing electron energy 70 eV and 1 mA intensity. The infrared spectra were taken with a Perkin—Elmer 457 apparatus using a KBr technique, the ultraviolet spectra with a ORD/UV-5 Jasco spectrometer in ethanol solutions. Alumina Reanal according to Brockmann (activity grade II) was

employed for column chromatography; the purity of alkaloids was checked on loose-layer chromatographic plates coated with a neutral alumina (activity grade VI) in a solvent system benzene—chloroform—ethanol 8 12 : 3.

Isolation of alkaloids

The drug (terminal twigs of *Buxus sempervirens* var. *argentea* HORT. ex. STEUD.) was collected at the end of October 1969 and 1970 in the Arboretum of the Slovak Academy of Sciences in Mlyňany. The dry leaves (2.1 kg) were removed from the woody parts, ground and macerated 5 × with acetic acid acidified (5%) aqueous methanol (50%, 50 l total). The organic solvent was distilled off under diminished pressure and the acid aqueous solution worked up as described earlier [6]. The mixture of alkaloids (49 g, 1.4%) was distributed into pH 6.5, 6.0, 5.0, 4.0, 3.0 McIlvain buffer solutions and 5% hydrochloric acid. The bases from the respective solutions were liberated by dilute ammonia and extracted with chloroform. Pure alkaloids constituting the individual pH portions were prepared by column or preparative t.l.c. chromatography. The principal alkaloids of this plant are cyclobuxine-D and cyclovirobuxine-D. The appearance of alkaloids in the individual pH fractions is seen in Table 1.

Characterization of the isolated alkaloids

Cyclovirobuxine-D: m.p. 212—214°C (acetone—dichloromethane), $[\alpha]_D^{21}$ +65° (*c* 1.01). This base was identified by means of its mass spectrum showing peaks at *m/e* 402 (M^+), M-15, M-30, M-56, 70, 58, 57, and 44, characteristic of methylamino substitutions at C-3 and C-20, infrared spectrum indicative of a hydroxyl (1038 and 3310 cm^{-1}), *gem*-dimethyl (1378 cm^{-1}) and cyclopropylmethylene (1458 cm^{-1}) groups and by comparison of these spectra with those of the authentic specimen.

Cyclobuxine-D: m.p. 231—232°C (acetone—dichloromethane), $[\alpha]_D^{22}$ +97° (*c* 1.00). The mass spectrum revealed the peak of the molecular radical ion at *m/e* 386 and a fragmentation typical of methylamino groupings at C-3 and C-20. The infrared spectrum of this base was superimposal with that of the authentic specimen. The mixed melting point did not display any depression.

Cycloprotobuxine-C: m.p. 192°C (acetone), $[\alpha]_D^{24}$ +69° (*c* 0.72 ethanol). The identity of this alkaloid was confirmed spectrometrically and on the basis of coincidence of all physicochemical constants with those of the authentic specimen. The mass spectrum showed the peak of the molecular radical ion at *m/e* 400, and a fragmentation at *m/e* M-15, M-44, M-72 characteristic of a dimethylamino group at C-20. The infrared spectrum was diagnostic of a cyclopropylmethylene (1450 and 3040 cm^{-1}), dimethylamino group (1260 and 2778 cm^{-1}) and a *sec*-amino group (3420 cm^{-1}). Signals in the p.m.r. spectrum paralleled the presence of groups identified by the above-mentioned spectral methods and evidenced protons of four *tert*-methyl groups at 0.97 (6H, s), 0.94 (3H, s), 0.78 (3H, s) and one *sec*-methyl group at 0.84 (3H, d, $J = 6$ Hz). All these data are in accordance with those reported for cycloprotobuxine-C [8].

Cycloprotobuxine-D: m.p. 129—131°C, $[\alpha]_D^{21}$ +108° (*c* 1.00). The mass spectrum indicative of methylamino groups at C-3 and C-20 proved also the molecular weight 386. The infrared spectrum of this base differed from that of cycloprotobuxine-C only slightly (lack of the typical vibration of dimethylamino group at 1260 and 2778 cm^{-1}).

Cyclobuxamine-H: amorphous, $[\alpha]_D^{22}$ +28° (*c* 0.9). *N*-Isopropylidene derivative: m.p. 245—247°C (decomposition, acetone). This base was identified on the basis of infrared spectrum showing bands of a cyclopropylmethylene grouping (1440 and 3020 cm^{-1}), a hydroxyl (1030 and 3400 cm^{-1}) and amine (3300 cm^{-1}) groups, an azomethine (1660 cm^{-1}) vibration band of the *N*-isopropylidene derivative and by the mixed melting point of the latter with an authentic specimen.

Buxtauine-M: m.p. 178°C (acetone), $[\alpha]_D^{24}$ +158° (*c* 0.96). Its mass spectrum with the molecular radical ion peak at *m/e* 371 was characteristic of one nitrogen-containing bases possessing a C-3

Table 1

Occurrence of alkaloids in the individual pH portions

Portion		Volume/fraction ml	Eluant	Fraction	Alkaloid	mg
pH	g					
6.5	12.5	150	Benzene	2—3	Cycloprotobuxine-C	93.0
			Benzene	5	Cycloprotobuxine-D	7.0
			Benzene + 0.5%MeOH	20—50	Cyclobuxine-D + cyclovirobuxine-D	1330.0
			Benzene + 1% MeOH	51—92	Cyclobuxamine-H	21.0
6.0	7.3	400	Benzene	1	Cycloprotobuxine-C	98.0
			Benzene	2	Cycloprotobuxine-D	7.0
5.0	5.5	15	Benzene + 2% MeOH	21	Buxtauine-M	18.0
			Benzene + 2% MeOH	22	B-426	2.0
4.0	3.5	60	Benzene + 20% LP	1	Cyclobuxoviridine-L	9.0
			Benzene + 20% LP	5	B-398	1.5
			Benzene	130	Irehine	13.0
3.0	4.0	20	Benzene + 30% LP	1—8	Cyclobuxoviridine-L	107.0
			Benzene	9—37	Cyclobuxophyllinine-M	15.0
			Benzene + 1% MeOH	37—73	Cyclobuxargentine-G	5.0

LP — light petroleum.

methylamino substitution and C-20 carbonyl. The intensity of the last two peaks of the fragmentation series at *m/e* 44, 57, 70 was markedly lowered indicating thus the presence of an exomethylene group at C-4 [12]. The identity was proved by comparing the spectra and also by a mixed melting point with an authentic specimen.

Irehine: m.p. 172°C (methanol), $[\alpha]_D^{22}$ −50° (*c* 1.02). This alkaloid was recognized by spectral means: the mass spectrum displayed a fragmentation pattern associated with the dimethylamino group at C-20, the infrared spectrum bands of a Δ^5-3β-OH grouping (1062 cm^{-1}). No depression of melting point was found on admixture of an authentic specimen.

Cyclobuxophyllinine-M: m.p. 178—179°C (benzene—methanol), $[\alpha]_D^{22}$ −42° (*c* 0.83). The mass spectrum of this alkaloid had a peak at *m/e* 369 (M^+) and a fragmentation pattern attributable to a methylamino group at C-3. The absorption at 343 nm (log ε 4.05) indicated the presence of a conjugated ketone, the infrared spectrum revealed bands of a carbonyl (1720 cm^{-1}), olefinic (1640 cm^{-1}), and a cyclopropylmethylene (1460 and 3045 cm^{-1}) vibrations. The obtained data compared with those reported for cyclobuxophyllinine-M [13].

Cyclobuxoviridine-L: b.t. 180—182°C (light petroleum), $[\alpha]_D^{23}$ −16° (*c* 0.90).

Cyclobuxargentine-G: m.p. 283°C (methanol—dichloromethane), $[\alpha]_D^{23}$ −51° (*c* 1.37).

Unidentified bases

B-426: m.p. 208—210°C (methanol—dichloromethane).

B-398: m.p. 197—199°C (benzene—methanol).

All spectra were recorded in the Department of Analytical Chemistry, Institute of Chemistry, Slovak Academy of Sciences (Head C. Peciar).

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