

Buxus alkaloids. XVIII.*
Alkaloids of *Buxus Harlandi* HANCE

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Received 21 May 1980

Seven steroid alkaloids were isolated and identified from the leaves of *Buxus Harlandi* HANCE. Cyclobuxine-D, cycloprotobuxine-D, cycloprotobuxine-C, buxamine-E, buxtauine-M, and buxpiine-K were those already known; the new alkaloid, the constitution and configuration of which was deduced from spectral data and corroborated by correlation, was denominated buxamine-B.

Из листьев *Buxus Harlandi* HANCE было изолировано и идентифицировано 7 стероидных алкалоидов. Известными являются: циклобуксин-D, циклопротобуксин-D, циклопротобуксин-C, буксамин-E, букстаунин-M и букспиин-K. Новый алкалоид, которого структуру и конфигурацию мы определили по спектральным данным и подтвердили корреляцией, был нами назван буксамин-B.

The mixture of alkaloids isolated from plants of the genus *Buxus* has been utilized in treatment of various diseases. Comparison showed that alkaloids present in the individual species differ from each other not only quantitatively, but also qualitatively. Bases present in the leaves of *B. Harlandi* have not been isolated so far and therefore, we included this species in our systematic studies.

Dried and ground leaves of *B. Harlandi* were extracted and the mixture of alkaloids obtained in this way was partitioned by extraction at various pH into separate portions from which the alkaloids were isolated by a procedure described in our previous papers [1, 2].

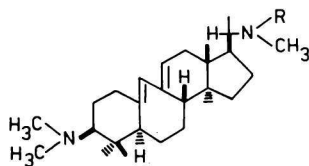
The pH 6.5 extract afforded two alkaloids of molecular weight 386. The structure of the first of them, deduced from spectral data, was verified by comparison with the specimen of cyclobuxine-D [3] (identical i.r. and mass spectra, optical rotations, and mixed melting point without depression). The second alkaloid of the same molecular weight showed in its mass spectrum peaks of fragment ions indicative of methylamino groups at C-3 (m/z 44, 57, 70) and C-20 (m/z $M - 15$, $M - 30$, and 58) [4]. Basing upon this fact, i.r. spectrum, and the

* For Part XVII see *Collect. Czech. Chem. Commun.* 42, 2549 (1977).

peak of molecular ion determined by high-resolution measurement at 386.3657 (for $C_{26}H_{46}N_2$ calculated 386.3661) this alkaloid could be identical with cycloprotobuxine-D. Its melting point and optical rotation disagreed with the data published for this alkaloid [5] and therefore, we prepared its N,N' -diacetyl and N,N' -dimethyl derivatives. The N,N' -diacetyl derivative, prepared by acetylation with acetic anhydride in pyridine, had the i.r., 1H -n.m.r., and mass spectra, and also the melting point in accord with the published data [5], the N,N' -dimethyl derivative, obtained by methylation according to *Eschweiler* and *Clarke's* method [6, 7] had melting point, optical rotation, and mass spectrum in line with the data reported for cycloprotobuxine-A (dimethylcycloprotobuxine-D) [8]. The discord between melting points and optical rotations of the native alkaloid we cannot so far rationalize.

From the pH 6.0 extract alkaloids cycloprotobuxine-C [9, 1] and amorphous buxamine-E were isolated, the first of which was identified in an analogous way as described with cyclobuxine-D. Since there was no specimen of buxamine-E available, two its derivatives, isopropylidenebuxamine-E and N -acetylbuxamine-E, were prepared; their spectral and physicochemical data did not differ from those reported [10]. Buxamine-E was found to be the principal alkaloid of leaves of this plant.

The extract of pH 5.0 contained buxtaurine-M and a new alkaloid of molecular formula $C_{27}H_{46}N_2$ (*I*), the mass spectrum of which displayed the peak of molecular radical ion at m/z 398.3648 (calculated 398.3661) and further peaks at m/z 383 ($M - 15$), 368 ($M - 30$), 355, 353, 253, 84, 71, and 58. The fragment ions at m/z 58, 71, and 84 were characteristic of a dimethylamino group at C-3 and those at m/z $M - 15$, $M - 30$, and 58 of a methylamino group at C-20 [4]. The absorption bands in the u.v. region at 238, 246, and 255 nm ($\log \epsilon$ 4.34, 4.39, and 3.74) were diagnostic of buxus alkaloid having a heteroannular conjugated diene system incorporated in its molecule [10]. Taking these results into account, this alkaloid was assigned the structure of 3-dimethylamino-20-methylamino-4,4,14-trimethyl-9(10 \rightarrow 19)abeo-5 α -pregna-9(11),10(19)-diene. To prove this proposal and to determine the configuration, two derivatives were prepared: acetylation with



I R = H

II R = CH_3CO

III R = CH_3

Fig. 1

acetic anhydride in pyridine afforded the *N*-acetyl derivative *II* (M^+ 440), methylation with methyl iodide the *N*-methyl derivative *III* (M^+ 412), the latter being identical with buxamine-A [11]. According to the convention adopted on the IUPAC symposium in Kyoto [12] this alkaloid should be denominated buxamine-B (Fig. 1).

Buxpiine-K and buxtauine-M were identified on the basis of spectral data and comparison with the authentic specimens [2, 13, 14] in the extract of pH 4.0; extracts of pH 3.0 and that obtained with 2% HCl contained a complex mixture from which no individuals could be separated.

Alkaloids isolated from the leaves of *B. Harlandi* HANCE and their amounts are listed in Table 1.

Table 1

Alkaloids isolated from buffer solutions

Total alkaloid content in		Alkaloid	Yield mg
pH	g		
6.5	11.34	Cyclobuxine-D	172
		Cycloprotobuxine-D	51
		Cycloprotobuxine-C	17
6.0	5.68	Cycloprotobuxine-C	75
		Buxamine-E	1120
5.0	6.14	Buxamine-B	55
		Buxtauine-M	170
		Buxtauine-M	30
4.0	2.96	Buxpiine-K	16
		—	—
3.0	2.56	—	—
2% HCl	1.37	—	—

Experimental

Melting points were determined on a Kofler stage, optical rotation was measured with a Perkin—Elmer, model 141, apparatus in chloroform (unless otherwise stated), the i.r. spectra with a Perkin—Elmer, model 457, instrument in KBr, the mass spectra with an AEI-MS 902 spectrometer, the proton magnetic resonance spectra of deuteriochloroform solutions containing tetramethylsilane with a 487-B Tesla apparatus operating at 80 MHz (δ scale, p.p.m.). For thin-layer chromatography on Al_2O_3 G (Woelm t.l.c.) coated plates dried at room temperature for 24 h following solvent systems were used: chloroform—benzene—ethanol 8:4:1 (S_1) and chloroform—benzene—ethanol 8:4:2 (S_2).

Extraction of the drug and isolation of alkaloids

The dried and ground leaves of *B. Harlandi* HANCE (1915 g), collected in September 1975 in the Botanic Garden of the Komenský University in Bratislava, were 6 times macerated at room temperature with methanol—water—acetic acid (19:19:2) for 24 h. The collected extracts (46.5 l) were concentrated under diminished pressure to 1.5 l. Polysaccharides were removed from the solution as a pale yellow precipitate by a successive addition of a 6-fold volume of ethanol with stirring; the filtrate was thickened to approximately the same volume and the sirupy solution was alkalinized with 20% ammonia to pH 10. The alkaloids, quantitatively extracted with chloroform (a negative reaction of the distillation residue with Mayer reagent), were distributed into portions employing extraction with buffer solutions. The respective pH 6.5, 6.0, 5.0, 4.0, 3.0 portions and that obtained with 2% HCl were worked up in a usual way and chromatographed on alumina (Reanal, neutral, activity grade IV) column, benzene and the mixture benzene—ethanol in various ratios being the eluent.

Characterization of the isolated alkaloids

Cyclobuxine-D: M.p. 240—245°C (ethanol), $[\alpha]_D^{22} = +98^\circ$ (*c* 0.5), R_f 0.10 (S_1), 0.19 (S_2); Ref. [3]: m.p. 245°C, $[\alpha]_D = +98$. EIMS (*m/z*): 386 (M^+), 371, 356, 328, 70, 58, 57, 44. The mixed melting point showed no depression.

Cycloprotobuxine-D: M.p. >360°C (dichloromethane), $[\alpha]_D^{23} = +47^\circ$ (*c* 0.45, ethanol), R_f 0.16 (S_1); Ref. [5]: m.p. 140—142°C, $[\alpha]_D^{18} = +112^\circ$ (chloroform). EIMS (*m/z*): 386 (M^+), 371, 356, 340, 330, 314, 70, 58, 57, 44.

N,N'-Diacetylcycloprotobuxine-D: Cycloprotobuxine-D (35 mg) dissolved in pyridine—acetic anhydride (1 ml each) was heated on a steam bath for 20 h. Pyridine and the excessing acetic anhydride were removed *in vacuo*, the residue (approx. 40 mg) was chromatographed on alumina (1 g). Elution with chloroform—ethanol (199:1) afforded *N,N'*-diacetylcycloprotobuxine-D. Melting point 273—276°C (acetone), $[\alpha]_D^{22} = +3.1^\circ$ (ethanol, *c* 0.64), R_f 0.85 (S_1); Ref. [5]: m. p. 276—278°C. EIMS (*m/z*): 470 (M^+), 455, 427, 100, 57. IR spectrum (CHCl_3): 1620 cm^{-1} ($\nu(\text{N—CO})$).

Cycloprotobuxine-A from *cycloprotobuxine-D*: Cycloprotobuxine-D (40 mg) dissolved in formic acid (85%, 1.5 ml) and formaldehyde (38%, 1.5 ml) was heated under reflux condenser on a steam bath for 4 h. The cooled mixture was diluted with water (10 ml), acidified with 5% HCl and extracted with ether. The aqueous layer was made alkaline with NH_4OH and extracted with chloroform (4 × 10 ml). The collected chloroform extracts were washed with water and the solvent was removed under diminished pressure. The dry residue (43 mg) was chromatographed on alumina (Woelm, basic, activity grade III, 2 g). Elution with benzene furnished cycloprotobuxine-A. Melting point 205—208°C (acetone), $[\alpha]_D^{24} = +70^\circ$ (*c* 0.35), R_f 0.78 (S_1); Ref. [7]: m.p. 206—207°C, $[\alpha]_D = +76^\circ$. EIMS (*m/z*): 414 (M^+), 399, 370, 343, 328, 84, 72, 71, 58.

Cycloprotobuxine-C: M.p. 200°C (acetone), $[\alpha]_D^{22} = +76^\circ$ (*c* 0.56, ethanol), R_f 0.50 (S_1); Ref. [8]: m.p. 200—202°C, $[\alpha]_D = +76^\circ$ (chloroform). EIMS (*m/z*): 400 (M^+), 385, 356, 328, 72, 70, 57, 44.

Buxamine-E: Amorphous, $[\alpha]_D^{22} = +32^\circ$ (c 0.73), R_f 0.12 (S_1). EIMS (m/z): 384 (M^+), 370, 341, 353, 84, 71, 58.

Isopropylidenebuxamine-E: Amorphous buxamine-E was dissolved in acetone (2 ml) and allowed to crystallize. Melting point 184°C (acetone), $[\alpha]_D^{22} = +56^\circ$ (c 0.56, ethanol), R_f 0.12 (S_1); Ref. [9]: m.p. 187°C , $[\alpha]_D^{20} = +48^\circ$ (chloroform). EIMS (m/z): 424 (M^+), 409, 253, 84, 71, 58. IR spectrum: 1660 cm^{-1} ($\nu(\text{C}=\text{N})$).

Acetylbuxamine-E: Buxamine-E (50 mg) dissolved in pyridine (1 ml) and acetic anhydride (1 ml) was left to stand at room temperature for 20 h, the excessing acetylation mixture was distilled off under reduced pressure and the residue (55 mg) chromatographed on alumina (1.5 g). Chloroform—ethanol (199:1) eluted the required derivative: m.p. $185\text{--}190^\circ\text{C}$ (acetone), $[\alpha]_D^{23} = +3.98^\circ$ (c 0.52), R_f 0.82 (S_1); Ref. [9]: m.p. 210°C , $[\alpha]_D^{20} = +5^\circ$. EIMS (m/z): 426 (M^+), 411, 383, 84, 71, 58, 44.

Buxamine-B: Rechromatography of the portion obtained at pH 5.0 on alumina (Woelm, basic, activity grade III) with benzene—ether (95:5) afforded the title alkaloid of m.p. 248°C , $[\alpha]_D^{23} = +61.4^\circ$ (c 0.57), R_f 0.4 (S_1). $^1\text{H-N.m.r.}$ spectrum: 0.70 (9H, s), 1.00 (3H, s) 4 *tert*- CH_3 groups; 1.03 (3H, d, $J = 15\text{ Hz}$) *sec*- CH_3 group; 2.27 (6H, s) dimethylamino group at C-3; 2.40 (3H, s) methylamino group at C-20; 5.50 (m), 5.91 (s) two olefinic protons of a heteroannular conjugated system.

Acetylbuxamine-B: Buxamine-B (18 mg) dissolved in pyridine:acetone (1:1, 1.4 ml) was left to stand at room temperature for 18 h, the excess of the acetylation mixture was distilled off *in vacuo*, and the residue (21 mg) chromatographed on alumina (Reanal, activity grade III, 1 g). Elution with benzene—ethanol (99:1) yielded the pure *II*, m.p. $186\text{--}190^\circ\text{C}$ (acetone), R_f 0.84 (S_1). EIMS (m/z): 440 (M^+), 397, 253, 100, 84, 71, 58.

Buxamine-A from buxamine-B: CH_3I (0.1 ml) was added to the solution of buxamine-B (12 mg) in dichloromethane (2 ml). The mixture was allowed to stand at room temperature for 20 h, the solvent was distilled off and the residue treated with 0.1 M methanolic KOH (1 ml). The mixture was concentrated, diluted with water and the product extracted with chloroform was worked up in a routine manner and chromatographed (alumina, 1 g). Elution with chloroform—ethanol (99.5:0.5) afforded *III*, m.p. $131\text{--}134^\circ\text{C}$ (acetone), $[\alpha]_D^{22} = +40^\circ$ (c 0.2), R_f 0.83 (S_1); Ref. [10]: m.p. 134°C , $[\alpha]_D^{22} = +40^\circ$. EIMS (m/z): 412 (M^+), 398, 397, 84, 72, 71, 58. The mixed melting point with buxamine-A revealed no depression.

Buxtaïne-M: M.p. $174\text{--}178^\circ\text{C}$ (ether), $[\alpha]_D^{22} = +150^\circ$ (c 0.68), R_f 0.55 (S_1). EIMS (m/z): 371 (M^+), 356, 313, 70, 57, 44, 43. Mixed melting point displayed no depression.

Buxpiine-K: M.p. $170\text{--}173^\circ\text{C}$ (ether), $[\alpha]_D^{22} = +156^\circ$ (c 0.53), R_f 0.74 (S_1). EIMS (m/z): 385 (M^+), 370, 342, 84, 71, 58, 43. Mixed melting point showed no depression.

Spectra were recorded in the Department of Physicoanalytical Methods, Institute of Chemistry, Slovak Academy of Sciences.

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Translated by Z. Votický