ALKALOIDS FROM *Buxus sempervirens* var. *bullata* KIRCHN.*

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Buxaminol-E was found to be the main alkaloid of *Buxus sempervirens* var. *bullata*; further bases identified in this plant were: cyclobuxine-D, cycloprotobuxine-C, cyclovirobuxeine-A, cyclovirobuxines-C and -D, and irehine. All the above-mentioned alkaloids were reported to be present also in other *Buxus* species. Moreover, two new bases cyclobullatine-A and buxaminol-B were separated and their structures proposed on the basis of spectral evidence and correlation with cyclobuxamine-H and buxaminol-E, respectively.

So far, not less than 87 structural formulas have been ascribed to alkaloids isolated from various species of *Buxus*[1–5], some of them being, however, assumed to be artifacts[6,7]. In this paper we wisch to report the isolation and structure elucidation of nine alkaloids obtained from *Buxus sempervirens* var. *bullata* KIRCHN.; the constitution of two additional amorphous bases, B-410 and B-412, could not be postulated because of separation difficulties.

The mixture of alkaloids, obtained in a usual manner[8] from dry ground material was fractionated according to their basicity. In the McIlvain pH 6·5 buffer extract cyclobuxine-D (ref.[9]) and cyclovirobuxine-D (ref.[10]) were identified together with a new alkaloid. This base had, according to the mass spectrum, molecular formula $C_{27}H_{46}N_2O$ (*M* calculated 414·3610, found 414·3615) and a fragmentation pattern indicative of a $C_{(3)}$-dimethylamino grouping (peaks at *m/e* 58, 71, 84) and $C_{(20)}$--methylamino substitution (peaks at *m/e* $M-15$, $M-30$ and 58) (ref.[11]). The high intensity of peaks at lower mass units excluded the presence of an exomethylene group at $C_{(4)}$ (*cf.*[11]). The IR spectrum with bands at 1108 and 3580 cm^{-1} (a hydroxy group), 3320 cm^{-1} (secondary amine), 1605 and 1660 cm^{-1} (a conjugated diene), 1390 cm^{-1} (gem. methyl groups) closely resembled that of buxaminol-E (ref.[12]). The presented spectral data fit the requirements for 3β-dimethylamino-20α-methylamino-4,4,14α-trimethyl-9(10 → 19)*abeo*-5α-pregna-9(11),10-dien-16α-ol (*I*). Basing upon convention[10] the trivial name buxaminol-B should be assigned to this new base. To evidence the stereochemistry assumed, buxaminol-B was methylated at $C_{(20)}$-

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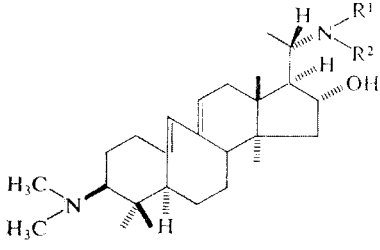
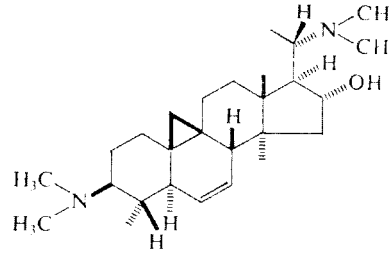
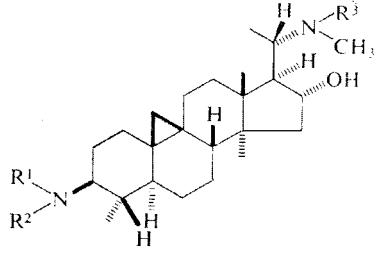
-nitrogen. The resulting N-methyl derivative was proved to be identical with buxaminol-A(II) prepared from buxaminol-E (*III*) by methylation. From the pH 6·0 buffer solution the already known[12] alkaloids buxaminol-E – the principal alkaloid of this plant – cyclobuxine-D, cyclovirobuxine-D, irehine[13] and bases B-410 and B-412 were separated. The pH 5·0 buffer extract yielded an additional amount of buxaminol-E and two other known[14,15] alkaloids cyclovirobuxine-C and cycloprotobuxine-C. The separation of bases present in the pH 4·0 extract afforded cyclovirobuxeine-A (ref.[16]) and a new base to which we proposed the name cyclobullatine-A (*IV*). Its molecular formula $C_{27}H_{46}N_2O$ was determined by high resolution mass spectrometry (*M* calculated 414·3610, found 414·3614). The base peak of this substance at *m/e* 58 together with other two peaks at *m/e* 71 and 84 of this fragmentation series were indicative of a dimethylamino grouping at $C_{(3)}$ whereas those at *m/e* $M-15$, $M-44$, $M-72$ and 72 of a dimethylamino substitution at $C_{(20)}$ (*cf*[11]). The IR spectrum revealed the presence of a secondary hydroxyl (1095 cm^{-1}), a tertiary amine (1260, 2760, 2778 and 2815 cm^{-1}), a cyclopropyl methylene groups (1450 cm^{-1} and 3030 cm^{-1}) and a secondary methyl group (1380 and 2958 cm^{-1}). The absorption maxi-



I, $R^1 = H$, $R^2 = CH_3$
II, $R^1 = R^2 = CH_3$
III, $R^1 = R^2 = H$



IV



V, $R^1 = R^2 = R^3 = H$
VI, $R^1 = R^2 = R^3 = CH_3$

SCHEME 1

mum at 204 nm (log ε, 3·63, was attributable to a isolated double bond at $C_{(6)}$. The enhancement of the logarithm of the molecular extinction coefficient from 3·17 to 3·63 might be due to a transannular interaction between the π-orbitals of the double bond and the π-like overlapping *p* orbitals of the cyclopropane ring in the distorted boat form of the ring B (ref.[17]). The presented data and the biogenetic consideration would allow to assign the structure 3β,20α-bis(dimethylamino)-4α,14α--dimethyl-9β,19-cyclo-5α-pregn-6-en-16α-ol to this alkaloid. To verify this suggestion, cyclobullatine-A was correlated with cyclobuxamine-H (*V*). The Eschweiler–-Clarke methylation of *V* furnished N,N,N'-trimethylcyclobuxamine-H [cyclobuxamine-A (*VI*)], which was found to be identical with dihydrocyclobullatine-A obtained by catalytical hydrogenation of *IV*.

EXPERIMENTAL

The melting points were determined on a Kofler micro hot-stage, the optical rotation in chloroform solutions with a Perkin-Elmer 141 apparatus in 1 cm cells. Mass spectra were measured with an MCh 1306 spectrometer (USSR) adapted for a direct introduction of the sample to the ionization chamber at the ionizing electron energy 70 eV and 1 mA intensity. High resolution mass measurements were taken with an AEI-MS 902 S spectrometer. Infrared spectra were recorded both with Perkin-Elmer 457 and Zeiss UR-10 apparatuses in KBr discs, ultraviolet spectra with an ORD/UV-5 JASCO spectrometer in ethanol. The alumina for column chromatography according to Brockmann (Merck) was of activity grade II; the purity of alkaloids was monitored by loose-layer chromatography on alumina Reanal, neutral, activity grade VI in a solvent system benzene–chloroform–ethanol 8 : 12 : 3.

Isolation of Alkaloids

The drug (terminal twigs of *Buxus sempervirens*, var. *bullata* KIRCHN.) was collected in October 1971 in the Arboretum of the Slovak Academy of Sciences in Mlyňany (southwestern Slovakia). Dry ground leaves (6·5 kg) were macerated five times with dilute methanol (50%) acidified with acetic acid (0·5%, 150 l total). The organic solvent was removed under diminished pressure and the acid aqueous solution of alkaloids was worked up as described previously[8]. The mixture of alkaloids (135·2 g, 2·08%) dissolved in chloroform (1 500 ml) was extracted with McIlvain pH 6·5, 6·0, 5·0, 4·0, 3·0 buffer solutions and 2% hydrochloric acid, respectively. The bases liberated from the separate solutions with dilute ammonia were extracted with chloroform and distributed by column chromatography over alumina. The mixture of alkaloids B-410 and B-412 was further separated by partition chromatography over Celite[18] in a solvent system benzene–dichloromethane–ethanol–water 10 : 14 : 1 : 0·3 using bromocresol red as a stationary phase indicator. The amount of the particular bases was not sufficient for the structure elucidation.

Characterization of the Isolated Alkaloids

Cyclovirobuxine-D: m.p. 221°C (dichloromethane–acetone), $[\alpha]_D^{21}$ +63° (*c* 0·98). The infrared, mass and proton magnetic resonance spectra were in accord with those of the authentic specimen.

Cyclobuxine-D: m.p. 247°C (dichloromethane–acetone), $[\alpha]_D^{22}$ +98° (*c* 1·01), was identified by spectral methods; the infrared, mass and proton magnetic resonance spectra were superimposable with those of the authentic sample.

Buxaminol-B: m.p. 225°C (acetone), $[\alpha]_D^{25}$ +20° (*c* 0·59, methanol). The peaks in the UV spectrum at λ_{max} (log ε) 236 nm (4·45), 246 nm (4·49), 255 nm (4·28), and 273 nm (2·68) were characteristic of a conjugated heteroannular diene[12].

Methylation of buxaminol-E *to buxaminol*-A: To buxaminol-E (221 mg) dissolved in dichloromethane (5 ml) methyl iodide (0·5 ml) was added and the solution was allowed to stand for 120 h at +5°C. The reaction mixture was evaporated *in vacuo*, 0·1M methanolic KOH (5 ml) was added and brought to a short simmer. After 2 h of standing the solvent was distilled off, the residue was suspended in water (50 ml) and extracted with dichloromethane. Yield 225 mg. The crude buxaminol-A was purified by preparative TLC (alumina Reanal activity grade VI) in a solvent system benzene–chloroform–ethanol 10 : 14 : 2 and crystallized from dichloromethane, m.p. 146—7°C; $[\alpha]_D^{22}$ +34° (*c* 1·0).

Methylation of buxaminol-B *to buxaminol*-A: The same procedure as described with buxaminol-E was applied to buxaminol-B (10 mg).

Buxaminol-E: amorphous, $[\alpha]_D^{22}$ +36° (*c* 0·5) was characterized by comparison of the UV and IR spectra with those of the authentic specimen. Also m.p. and specific rotation of the N-isopropylidene derivative [m.p 205—206°C (acetone), $[\alpha]_D^{22}$ +95° (*c* 0·6)] were in agreement with those reported[12].

Irehine: m.p. 173°C (acetone), $[\alpha]_D^{24}$ −46° (*c* 0·9) was identified according to the characteristic mass spectrum and by an undepressed mixed melting point with the authentic specimen.

TABLE I

Separation of Alkaloids

pH Extract (g)	Alumina, g (fractions, ml)	Fractions No	Eluent	Alkaloids	Amount mg
6·5	500	40—63	C_6H_6	cyclovirobuxine-D	1 698·0
(16·5)	(200)	69—77	$CHCl_3$	cyclobuxine-D	786·8
		90	$CHCl_3$ + + 5% C_2H_5OH	buxaminol-B	30·0
6·0	500	15—38	C_6H_6	irehine	46·0
(16·7)	(200)	60	$CHCl_3$	cyclobuxine-D, cyclovirobuxine-D	240·0
		65—74	C_2H_5OH	buxaminol-E	2 330·0
		75—82	C_2H_5OH + + 50% CH_3OH	B-410, B-412	670·0
5·0	420	13—39	C_6H_6	cycloprotobuxine-C	572·6
(14·0)	(150)	40	$(C_2H_5)_2O$	cyclovirobuxine-C	631·6
		60—76	C_2H_5OH	buxaminol-E	68·0
4·0	300	7—9	$(C_2H_5)_2O$	cyclovirobuxeine-A	15·1
(10·3)	(150)	27—30	C_2H_5OH	cyclobullatine-A	17·9

Cyclovirobuxine-C: m.p. 200°C (acetone–methanol), $[\alpha]_D^{21}$ +62° (*c* 1·0). The identity of this alkaloid was confirmed by spectral means: the fragmention pattern and ^{1}H-NMR spectrum coincided with those reported[14].

Cycloprotobuxine-C: m.p. 210—211°C (acetone), $[\alpha]_D^{24}$ +68° (*c* 0·5, ethanol). The ^{1}H-NMR and mass spectra were in agreement with those of cycloprotobuxine-C isolated previously from *Buxus wallichiana* BAILLON (ref.[15]).

Cyclovirobuxeine-A: m.p. 217—218°C (acetone), $[\alpha]_D^{23}$ −85° (*c* 1·0) almost accorded with those reported[16] [m.p. 220°C, $[\alpha]_D$ −87° (*c* 1·0)]. Decisive for identification of the base was the ^{1}H-NMR spectrum showing peaks indicative of the cyclopropane ring, four tertiary methyl groups, one secondary methyl group, two olefinic protons in addition to signals due to a secondary alcohol and two dimethylamino groups.

Cyclobullatine-A: m.p. 275°C (acetone), $[\alpha]_D^{22}$ −99° (*c* 0·46, ethanol).

Dihydrocyclobullatine-A: m.p. 215—217°C (ethanol), $[\alpha]_D^{25}$ +37° (*c* 0·45). Cyclobullatine-A (5 mg) dissolved in acetic acid (10 ml) was hydrogenated over Adams catalyst (15 mg) overnight. Consumption of hydrogen 3·5 ml at room temperature. The reaction mixture was diluted with water (40 ml), basified with ammonia and extracted with dichloromethane. Physical constants of dihydrocyclobullatine-A coincided with those of cyclobuxamine-A and the mixed melting point of both derivatives revealed no depression.

Cyclobuxamine-A: Cyclobuxamine-H (33·6 mg) was methylated with formic acid and formaldehyde according to[19]. Physical data were in accordance with those reported. The configuration of the $C_{(4)}$ methyl group in cyclobuxamine-H has later been proved[20] to be α, *i.e.* opposite as originally published[19].

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REFERENCES

1. Černý V., Šorm F. in the book: *The Alkaloids* (R. H. F. Manske, Ed.), Vol. IX, p. 305. Academic Press, New York 1967.
2. Tomko J., Votický Z. in the book: *The Alkaloids* (R. H. F. Manske, Ed.), Vol. XIV, p. 32. Academic Press, New York 1973.
3. Döpke W., Härtel R.: Z. Chem. *13*, 135 (1973).
4. Döpke W., Härtel R.: Z. Chem. *13*, 178 (1973).
5. Shamma M., Georgiev V. S., Miana G. A., Khan F. S.: Phytochemistry *12*, 2051 (1973).
6. Votický Z., Paulík V.: Chem. Zvesti *26*, 376 (1972).
7. Nakano T., Terao S., Saeki Y.: J. Chem. Soc. *C*, *1966*, 1412.
8. Bauerová O., Votický Z.: Pharmazie *28*, 212 (1973).
9. Brown K. S. jr., Kupchan S. M.: J. Amer. Chem. Soc. *84*, 4590 (1962).
10. Brown K. S. jr., Kupchan S. M.: Tetrahedron Lett. *1964*, 2895.
11. Dolejš L., Hanuš V., Votický Z., Tomko J.: This Journal *30*, 2869 (1965).
12. Stauffacher D.: Helv. Chim. Acta *47*, 968 (1964).
13. Truong-Ho M., Monseur X., Khuong-Huu Q., Goutarel R.: Bull. Soc. Chim. Fr. *1963*, 2332.
14. Khuong-Huu F., Magdeleine M. J.: Ann. Pharm. Fr. *28*, 211 (1970).

15. Vassová A., Tomko J., Votický Z., Beal L. J.: Pharmazie *25*, 363 (1970).
16. Khuong-Huu-Lainé F., Magdeleine M. J., Bisset N. G., Goutarel R.: Bull. Soc. Chim. Fr. *1966*, 758.
17. Nakano T., Terao S.: J. Chem. Soc. *1965*, 4512.
18. Brown K. S. jr, Kupchan S. M.: J. Chromatogr. *9*, 71 (1962).
19. Brown K. S. jr, Kupchan S. M.: J. Amer. Chem. Soc. *86*, 4430 (1964).
20. Nakano T., Votický Z.: J. Chem. Soc., *C 1970*, 590.

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