BUXOZINE-C, A NOVEL TYPE OF BUXUS ALKALOID*

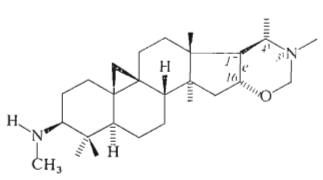
Z.VOTICKÝ[a], L.DOLEJŠ[b], O.BAUEROVÁ[a] and V.PAULÍK[a]

[a] *Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague*

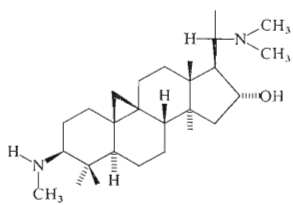
Received January 18th, 1977

The steroid alkaloid of a novel type, $C_{27}H_{46}N_2O$, isolated from *Buxus sempervirens* L. has been designated buxozine-C. Its structure has been deduced on the basis of spectral evidence and its configuration determined by correlation with cyclovirobuxine-C; the semisystematic name of this minor alkaloid is (4′*S*)-3′,4,4,4′,14α-pentamethyl-3β-methylamino-9,19-cylo-5α-androstano-[16β,17α-*e*]tetrahydro-1,3-oxazine. It is the first buxus alkaloid having a tetrahydrooxazine heterocycle fused in positions 16α,17β of the steroid backbone.

Differently substituted mono- and dibasic alkaloids with a cyclopropane ring in positions 9,19 or without it, with a seven-membered ring B, or a tetrahydrooxazine heterocycle attached to positions 3,4 have been reported as yet[1,2]. Also anomalous bases without substituents in positions 4 or 14 have been described[3,4]. The new alkaloid of molecular formula $C_{27}H_{46}N_2O$ (*I*), separated from the "strong bases" fraction[5] of *Buxus sempervirens* L. does not belong to any of the afore-mentioned types.



I



II

Its IR spectrum exihibited vibrations of a C—O—C bond (1 100 cm^{-1}), a cyclopropyl methylene (1 451 and 3 038 cm^{-1}), gem-methyl groups (1 190 and 1380 cm^{-1}, doublet), sec-amino and tert-amino groups (3 300 and 1 155 cm^{-1}), respectively. The ^{1}H-NMR spectrum (on the δ scale in ppm) showed the presence of protons ascribable

* Part XVII in the series *Buxus Alkaloids*; Part XVI: Phytochemistry, in press.

to a cyclopropyl methylene group at 0·33 and 0·60 (2 H, d, J = 4 Hz), four tert-methyls at 0·77, 0·87, 0·96 and 1·20 (4 × 3 H, ss), a sec-methyl at 1·10 (3 H, d, J = = 6·5 Hz), methylamino groups at $C_{(3)}$ at 2·29 (3 H, s) and $C_{(4')}$ at 2·45 (3 H, s) and a methylene group between two heteroatoms at 4·12 and 4·30 (2 H, q centered at 4·21, J = 7·5 Hz).

The mass spectrum (Fig. 1*a*) displayed a peak of the molecular ion at 414·3614 (for $C_{27}H_{46}N_2O$ calculated 414·3610) and after deuterium labelling in the ion source with $[O^2H]$ ethanol the occurrence of one active hydrogen. The fragmentation pattern of alkaloid *I* substantially differed from the character of other dibasic buxus alkaloids, *e.g.* from the spectrum of the structurally related cyclovirobuxine-C (*II*), (Fig. 1*b*). The fragmentation series characteristic of a monomethylamino substitution at $C_{(3)}$ (ref.[6]) at m/e 44 (*a*, C_2H_6N), 57 (*b*, C_3H_7N) and 70 (*c*, C_4H_8N), which remained unchanged, was manifested by an extraordinary high intensity of peaks. In the spectrum of the deuterated compound these peaks were shifted, in accordance with the formulation, by one mass unit. Further ions shifted after labellation, and containing therefore nitrogen atom at $C_{(3)}$, occurred at m/e 288 (*d*, $C_{20}H_{34}N$), 232 (*e*, $C_{16}H_{26}N$) and 219 ($C_{15}H_{25}N$); their appearance in the spectrum proves the presence of a gem-dimethyl group at $C_{(4)}$, whereas the fragment *b* indicates the presence of a cyclopropane ring attached to carbons $C_{(9)}$ and $C_{(10)}$ (ref.[6,7]). The peak at m/e 342 (*f*, M − $C_4H_{10}N$), unaltered after labellation, corresponds to an ion resulting

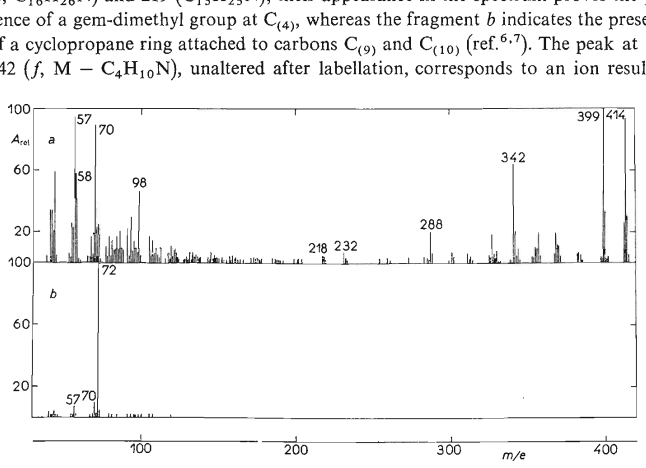
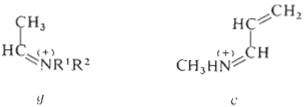


FIG. 1

Mass Spectra

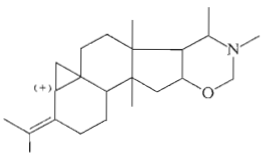
a Bar graph of buxozine-C, *b* bar graph of cyclovirobuxine-C. In the bar graph of cyclovirobuxine-C (Fig. 1*b*) the peaks at m/e 416 (M^+) 401, 372, 328 were erroneously omitted. Their intensities were 4, 3, 4 and 2% resp., of the base peak.

from the elimination of a methylpropylamine radical containing an acid hydrogen from the ionized molecule.

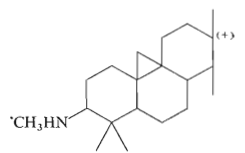


g *c* *b* *a*

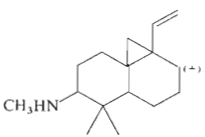
m/e 44: $R^1 = R^2 = H$
m/e 58: $R^1 = H$, $R^2 = CH_3$
m/e 72: $R^1 = R^2 = CH_3$



f



d



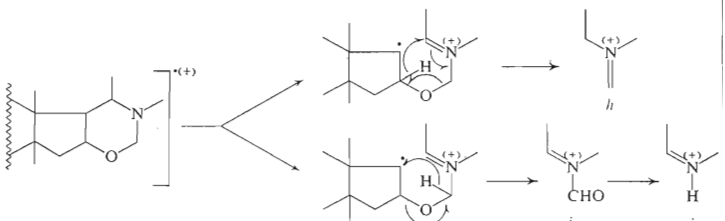
e

k

The intensity of the species M − 15, which constitutes the base peak of the spectrum, suggested that the cleaved methyl radical originated from α-position to $N_{(b)}$ atom. This phenomenon indicates the presence of an α-aminoethyl residue diagnostic of dibasic buxus alkaloids hitherto known. The presence of a free α-aminoethyl side chain is to be rejected on the basis of comparison of the mass spectrum of buxozine-C with that of cyclovirobuxine-C. The nitrogen-containing side chain, bound in dibasic buxus alkaloids at $C_{(17)}$, is represented by an intense peak of the ion *g* at *m/e* 44, 58, or 72 depending on the number of methyl substituents. The fragment *g* (*m/e* 72: $R^1 = R^2 = CH_3$) usually bears the most part of the total ion flow[6,8]. In the mass spectrum of substance *I* no peak, however, reaches the presumed intensity. The number of loci of unsaturation, following from the molecular formula, suggests the side chain to form a ring. The biogenetic view favourizes the closure through

the oxygen atom frequently attached to $C_{(16)}$ in buxus alkaloids. The postulated formulation *I* is in accordance with the number of acid hydrogens found in the molecule. The cyclic structure rationalizes the weakened influence of the $N_{(b)}$ atom on the fragmentation pattern of the substance, this being manifested by the substantially enhanced intensity of the molecular peak and the increased abundance of fragments containing the $N_{(a)}$ atom.

To verify the presumed structure, we utilized the already known[9] specificity of hydrogenolysis of a substituted tetrahydrooxazine ring to the corresponding amino alcohol. The reduction of *I* with lithium aluminium hydride afforded a substance identical with the authentic specimen of cyclovirobuxine-C (*II*). The obtained product was identified by physical constants and mass spectrum (Fig. 1*b*). It was thereby confirmed not only the correctness of the proposed structure, but also the spatial arrangement of the alkaloid *I*, (4′*S*)-3′,4,4,4′,14α-pentamethyl-3β-methylamino-9,19-cyclo-5α-androstano[16β,17α-*e*]tetrahydro-1,3-oxazine, which we denominated buxozine-C.



SCHEME 1

The fragmentations triggered by the tetrahydro-oxazine grouping could be plausibly rationalized by structure *I*. The amino species at *m/e* 72 (*h*, $C_4H_{10}N$) and 58 (*j*, C_3H_8N) of the labelled substance were preserved and obviously, they were formed after the preceding fission of the bond $C_{(17)}$—$C_{(4')}$ (Scheme 1). Although the corresponding metastable peak for the transition did not appear in the spectrum we presume that the fragment *j* was generated from the ion *i* (*m/e* 86, C_4H_8NO, without shift in the deuterated compound). The ion *i* evidences the mutual proximity of the $N_{(b)}$ and oxygen atoms in the molecule of buxozine-C. There is also the complemental ion of the fragment *i* at *m/e* 328 ($M - C_4H_8NO$) in the mass spectrum; this is with the deuterated substance shifted by one mass unit.

The species *k* at *m/e* 98 (C_5H_8NO)* formed during further fragmentation of the ion M − CH_3, is in accordance with the proposed structure of buxozine-C.

Among peaks of lower intensity found in the higher region of the spectrum we identified by high resolution measurement fragments M − 30 and M − 45 formed from the molecular radical ion by the stepwise loss of formaldehyde molecule and methyl radical.

EXPERIMENTAL

The melting points were determined on a Kofler micro hot-stage, the optical rotation of a chloroform solution with a Perkin-Elmer polarimeter, model 141, in 1 cm cells. The IR spectra were measured with a Perkin-Elmer spectrophotometer, model 457, in KBr discs, the ^{1}H-NMR spectrum with a Tesla BS 487 B apparatus operating at 80 MHz in deuteriochloroform, tetramethylsilane being the internal reference substance. The mass spectra were recorded with an AEI-MS 902 spectrometer at the following operating conditions: ion source temperature 150—160°C, electron voltage 70 eV, ionizing current 500 μA and accelerating voltage 8 kV. Alumina for column chromatography Merck basic was of activity grade II, for loose-layer TLC Reanal neutral, activity grade VI. Solvent system benzene–chloroform–ethanol 8 : 12 : 3, visualization with Dragendorf reagent.

Buxozine-C: "Strong bases"[5] (200 g) dissolved in chloroform (1 000 ml) were gradually extracted with McIlvain pH 6·5, 6·0, 5·0, and 4·0 buffer solutions and 2% HCl and worked up as already described[10]. The portion of pH 4·0 (30 g) was chromatographed over alumina (900 g) with solvents in an eluotropic order according to Reichstein[11]. The alkaloid crystallizing from the 32nd ether fraction (79 mg) had m.p. 137°C (ether), $[\alpha]_D^{21}$ +65° (*c* 0·6), R_F 0·8.

Reduction of buxozine-C to cyclovirobuxine-C: A solution of buxozine-C (10 mg) in ether (7 ml) was added to a suspension of $LiAlH_4$ (10 mg) in ether (10 mg) under stirring at room temperature; stirring was continued for 16 h. The mixture gave after work-up 9·4 mg of a substance, m.p. 201°C (acetone–hexane, the mixed m.p. with the authentic specimen of cyclovirobuxine-C revealed no depression), $[\alpha]_D^{22}$ +49° (*c* 0·85), R_F 0·60. The IR and mass spectra of the hydrogenolysis product coincided with those of cyclovirobuxine-C.

The IR *and* ^{1}H-NMR *spectra were measured in the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, the mass spectra in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.*

* The ion at *m/e* 98 is a 4 : 1 doublet; 20% of the peak height is due to the fragment $C_6H_{12}N$.

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Translated by author (Z. V.).