BUXALINE-C FROM Buxus sempervirens

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Continuing a study of the alkaloids of Buxus sempervirens cultivated in Tashkent [1], from the mixture of bases by separation according to their basic strength on a polybuffer apparatus [2] we have isolated a new alkaloid – buxaline-C – with the composition $C_{29}H_{50}N_2O$ (I), mp 230-232°C (ethanol), $[\alpha]_D$ + 29.41°.

The IR spectrum of (I) shows absorption bands at (cm^{-1}) 3050, 1450 (methylene of a cyclopropane ring) [3] and 1645 (N-acetyl). The NMR spectrum has the singlets of tertiary methyl groups, a $N-CO-CH_3$ group, a $N(CH_3)_2$ group, and a N-methylacetamide group, and a doublet from a secondary methyl group (Table 1). The mass spectrum has the main peaks of ions with m/e 44, 58, 70, 71, 72 (100%), 84, 85, 427, 442 M^+.

The acid hydrolysis of alkaloid (I) gave a base with the composition $C_{27}H_{48}N_2$ (II), mp 208-210°C, $[\alpha]_D$ + 127.42°, and acetic acid (paper chromatography) [4]. The IR spectrum of (II) lacked the absorption bands at 1645 cm^{-1} characteristic for a N-acetyl group. In the NMR spectrum, the signal from the ($N-CO-CH_3$) protons had disappeared and a new signal had appeared in the form of a singlet at 2.38 ppm ($-NH-CH_3$).

Consequently, alkaloid (I) is the N-acetyl derivative of the base (II). The acetylation of (II) gave (I). The base (II) forms a N-methyl derivative (III) and a N-benzoyl derivative (IV).

The mass-spectrometric fragmentation of the base (II) takes place similarly to that of cycloprotobuxine C (V) [5], but differs from that of (V) by the intensity of the peaks of the ions with m/e 42 (29%), 44 (27), 57 (30), 69 (22), 71 (20), 72 (100), 83 (20), 84 (12), 85 (19), 97 (18), 113 (14), 356 (20), 385 (12), 400 (36%) M^+. The maximum peak of the ion with m/e 72 in the mass spectrum of (I) and (II) arises as the result of the cleavage of the bond between C_{17} and C_{20} and confirms the presence of a dimethylamino group in position C_{20} in a pregnane nucleus [6, 7]. The peak of the ion with m/e 44 is formed by the cleavage of the C_2-C_3 and C_3-C_4 bonds and shows the presence of a $-NH-CH_3$ group at C_3 [5].

The physicochemical constants of the alkaloid (I) are close to those of the acetylated derivative of cycloprotobuxine C (VI) [8]. However, a marked difference is observed in the values of the specific rotations of the base (II) and of cycloprotobuxine C (V), and also between the methylated product (III) and the methylated derivative of cycloprotobuxine C (cycloprotobuxine A) (VII) [9]. In its melting point and the sign and magnitude of its specific rotation, the benzoyl derivative of (II) differs markedly from the benzoyl derivative of cycloprotobuxine C (VIII) [8]. The chemical shifts of the protons of the tertiary and secondary methyl groups of (II) are almost the same as those of cycloprotobuxine C (V) [9]. A mixture of (II) with cycloprotobuxine C (kindly given to us by I. Tomko) showed a depression of the melting point [10, 11].

In a comparison of the chemical shifts of the protons of the tertiary and secondary methyl groups of (II) and (III), an upfield displacement of the chemical shifts of the secondary methyl protons in (III) by 9 Hz is observed. Consequently, the alkaloid (II) apparently differs from cycloprotobuxine C by the configuration of the asymmetric center at C_{20}. On the basis of the facts given, buxaline-C (I) has the most probable structure and configuration of 3β-acetylmethylamino-20β-dimethylamino-4,4,14α-trimethyl-9β,19-cyclo-5α-pregnane.

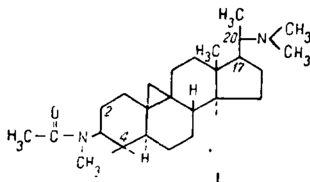

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TABLE 1

Alkaloid	Composition	mp, °C	$[\alpha]_D$, deg (CHCl$_3$)	NMR spectrum, δ
I	$C_{29}H_{50}N_2O$	230–232	+29,4	1,21 (s 6H, CH_3), 0,87, 0,78 (C, 6H, CH_3); 0,84 (d, 3H, CH_3; J=6 Hz); 2,85 (CH_3—CO—N—CH_3); 2,21 [N $(CH_3)_2$], 2,07 (N—CO—CH_3).
VI	$C_{29}H_{50}N_2O$	227–230	+15	—
II	$C_{27}H_{48}N_2$	208–210	+127,4	0,92 (s, 6H, CH_3); 0,89, 0,72 (C, 6H, CH_3) 0,78 (d, 3H, CH_3; J=6 Hz); 2,38 (N—CH_3); 2,13 [N$(CH_3)_2$]
V	$C_{27}H_{48}N_2$	200–202	+80	0,96 (s, 6H, CH_3); 0,93, 0,73 (s, 6H, CH_3); 0,83 (d, 3H, CH_3; J=6 Hz); 2,46 (N—CH_3); 2,20 [N $(CH_3)_2$].
IV	$C_{34}H_{52}N_2O$	260–262	—50,82	—
VIII	$C_{34}H_{52}N_2O$	218–220	+32	—
III	$C_{28}H_{50}N_2$	204–206	+5,1	0,94 (s, 6H, CH_3); 0,88, 0,76 (s, 6H, CH_3); 0,84 (d, 3H, CH_3; J=6 Hz) 2,25; 2,20 [12H, 2N $(CH_3)_2$].
VII	$C_{28}H_{50}N_2$	207	+75	0,97 (s, 6H, CH_3); 0,93, 0,80 (s, 6H, CH_3); 0,93 (d, 3H, CH_3; J=6 Hz); 2,30; 2,20 [12H, 2N $(CH_3)_2$].

Note: s) singlet; d) doublet.

EXPERIMENTAL

The homogeneity of the substances was checked by chromatography in a thin layer of silica gel Q in the butan-1-ol–acetic acid–water (10 :1 :3) system. The IR spectra were taken on a UR-20 spectrometer (KBr), the NMR spectra on a JNM-4H-100 MHz instrument (HMDS, $CDCl_3$, δ scale), and the mass spectra on an MKh-1303 instrument fitted with a glass system for the direct introduction of the sample into the ion source. The specific rotations of all the compounds were determined in chloroform.

Buxaline-C (I). The material (6.5 g) obtained from the aqueous fraction from the polybuffer separation of the alkaloids of *Buxus sempervirens* [2] was chromatographed on a column of alumina (activity grade II) (1 :100) and was eluted with chloroform–ethanol (9 :1), 10-ml fractions being collected. By treating the combined fractions 75-105 with ethanol, 0.4 g of buxaline C, $C_{29}H_{50}N_2O$, was obtained with mp 230-232°C (from ethanol), $[\alpha]_D$ + 29.4° (c 0.34); R_f 0.61, M^+ 442 (mass spectrometrically).

Hydrolysis of Buxaline-C to (II). A mixture of 0.12 g of (I), 25 ml of methanol, and 19 ml of 5% sulfuric acid was boiled for 8 h. After evaporation of the methanol in vacuum, the mixture was boiled for another 7 h. The solution was made alkaline with ammonia and extracted with methylene chloride. This yielded 0.1 g of the hydrolysis product (II) with the composition $C_{27}H_{48}N_2$, mp 208-210°C (from ethanol), $[\alpha]_D$ + 127.42° (c 0.481); R_f 0.30, M^+400 (mass spectrometrically).

N-Methyl Derivative of (II) (III). A mixture of 0.025 g of (II) was methylated by Hess's method [12]. This gave 0.027 g of the N-methyl derivative (III) with the composition $C_{28}H_{50}N_2$, mp 204-206°C (from ethanol), $[\alpha]_D$ + 5.1° (c 1.62), R_f 0.1, M^+ 414 (mass spectrometrically).

N-Benzoyl Derivative of (II) (IV). A solution of 0.021 g of (II) in 1 ml of pyridine was cooled, and 0.20 ml of benzoyl chloride was added dropwise and the mixture was allowed to stand at room temperature for 19 h. Then 30 ml of water was added and the product was extracted with benzene. This gave 0.019 g of the N-benzoyl derivative (IV) with the composition $C_{34}H_{52}N_2O$, mp 260-262°C (from ethanol). $[\alpha]_D$−50.82° (c 1.22), R_f0.55 M^+504 (mass spectrometrically). IR spectrum: ν_{max} 1635 cm^{-1} (– N–COC_6H_5).

SUMMARY

A new alkaloid buxaline-C has been isolated from *Buxus sempervirens* cultivated in Tashkent.

The results of a comparison of the physicochemical properties of the products of the hydrolysis of buxaline-C and those of cycloprotobuxine C have enabled the structure and configuration of buxaline-C to be determined as 3β-acetylmethylamino-20β-dimethylamino-4,4,14α-trimethyl-9β,19-cyclo-5α-pregnane.

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PROTAMINES

A STUDY OF THE AMINO-ACID SEQUENCE OF STURINE B

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We have previously described the isolation of sturine B and the determination of its amino-acid composition and of the N- and C-terminal amino acids [1]. In the present paper we consider the hydrolysis of sturine B by trupsin, the fractionation of the mixture of peptides obtained, and the determination of their structure, and also the study of the N- and C-terminal sequences of sturine B with the aid of leucine aminopeptidase and carboxypeptidases A and B.

The investigations of the primary structure of a series of protamines have shown that in their molecules the neutral amino acids are arranged in pairs or even in triplets [2]. Thus is particularly characteristic for the hydroxy amino acids serine and threonine.

Sturine B contains the 18-19 arginine residues, one histidine residue and a fairly limited set of neutral amino acids: Ala_1, Gly_2, Ser_2, Pro_1, Glu_1. To determine their positions in the molecule of sturine B it appeared appropriate to use trypsin hydrolysis, in which the bonds formed by residues of neutral amino acids remain unchanged. The presence in the sturine B molecule of one proline residue, which considerably retards the hydrolysis of the bonds adjacent to it permitted us to hope to obtain fairly large fragments.

The combination of trypsin hydrolysis with the hydrolysis of sturine B by leucine aminopeptidase and carboxypeptidase made it possible to determine the bulk of the sequence of aminoacids in the protein.

In a preliminary experiment on the kinetics of the hydrolysis of sturine B by trypsin with the aid of the ninhydrin reaction, it was shown that the cleavage of the peptide bonds is practically complete after 6 h (Fig. 1).

To isolate the peptides formed, the 6-h hydrolyzate of the hydrochloride of sturine B was separated by ion-exchange chromatography on Amberlite CG-50 resin, using elution with Na borate buffer mixture by Ando's method [3], modified by ourselves. We lowered the pH of the buffer mixture to 7.4, since under the conditions of Ando's method (pH 8.0) the peptides are not completely absorbed on the resin; the elution was performed by increasing the NaCl concentration gradient-wise and not stepwise, which ensured a fairly good separation of the peptides.

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