PSEUDOCYCLOBUXIN-D FROM Buxus sempervirens

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We have previously [1] reported the identification of an alkaloid with mp 235-237°C isolated from B. sempervirens by comparison with an authentic sample of cyclobuxin-D [2-4]. Continuing a study of the alkaloids of B. sempervirens cultivated in the Shreder experimental station, we have found that it contains 1.41% of a mixture of bases. By separating the ethereal alkaloids according to their solubilities, we obtained a new alkaloid pseudocyclobuxin-D with the composition $C_{25}H_{42}N_2O$ (I). Its IR spectrum has the characteristic absorption bands of a methylenecyclopropane ring, and terminal methylene, hydroxy, and secondary amino groups. The NMR spectrum shows the following resonance signals: the singlets of two tertiary methyl and two $N-CH_3$ groups, the doublets of terminal methylene and secondary methyl groups, and the multiplet of a methine proton geminal to a hydroxy group ($-CH-OH$), which shows the 16β proton of the series of Buxus alkaloids [5]. The mass-spectrometric decomposition of (I) takes place similarly to the decomposition of cyclobuxin-D (II) [3]. In the mass spectrum of (I) the peak of an ion with m/e 44 shows the presence of a 3-methylamino group and also of an exomethylene group at C_4 and of the methylene of a cyclopropane ring at $C_9 - C_{10}$; the peak of an ion with m/e 58 is characteristic for a 20α-methylamino group of a side chain of the pregnane nucleus [6, 7].

Pseudocyclobuxin-D undergoes Hess methylation, forming a N,N'-dimethyl derivative (pseudocyclobuxin-A) (III) in the IR spectrum of which the absorption bands of NH groups are absent. The NMR spectrum of (III) include the signals of two $N(CH_3)_2$ groups and in the mass spectrum the peaks of ions with m/e 71, 72, and 84, showing the complete methylation of the amino group in pseudocyclobuxin-D [6, 8], are observed.

The acetylation of (I) with acetic anhydride in pyridine forms N,N',O-triacetylpseudocyclobuxin-D (IV), in the IR spectrum of which the absorption bands of hydroxy and amino groups have disappeared. In the NMR spectrum of (IV), the appearance of resonance signals at 4.95, 2.09, 2.00, and 1.93 ppm confirms

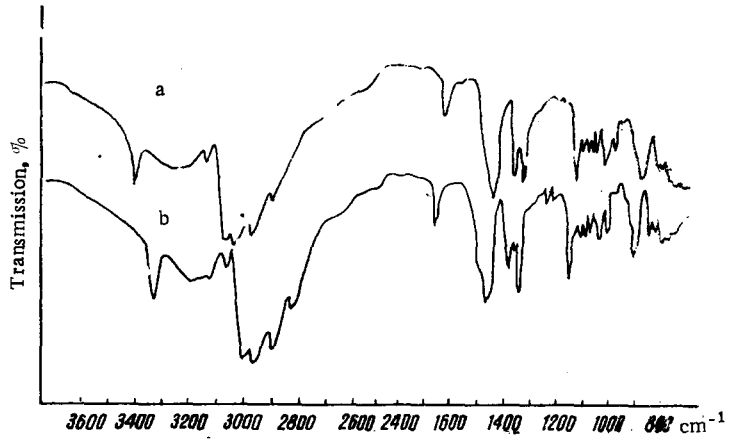

Fig. 1. IR spectrum of cyclobuxin-D (a) and pseudocyclobuxin-D (b).

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TABLE 1. Spectral Characteristics and Constants of Compounds (I) and (II) and Their Derivatives

Alkaloid	Composition	mp, °C	$[\alpha]_D$, deg	IR spectrum, cm^{-1}	NMR spectrum, δ	Mass spectrum (main fragments, %)
I	$C_{25}H_{42}N_2O$	229—231	+89,64	3312, 3155 (OH, NH) 2935 (CH_2, CH_3), 3048, 1452 (methylenecyclopropane ring) 1648, 910 (ring CH_2)	4,77; 4,54 (d, 2H, ring CH_2-CH_2; J 1 Hz); 3,97 (m, 1H, CH—OH); 2,44; 2,38 (C, 6H, 2N—CH_3); 1,09; 0,94 (C, 6H, CH_3); 1,05 (d, 3H, CH_3; J=7 Hz)	$41_{(5)}$, $43_{(8)}$, **$44_{(100)}$**, $45_{(6)}$, $55_{(4)}$, $56_{(5)}$, $57_{(1)}$, $58_{(1)}$, $70_{(2)}$, $312_{(9)}$, $328_{(4)}$, $330_{(5)}$, $340_{(22)}$, $355_{(25)}$, $356_{(10)}$, **$357_{(3)}$**, $370_{(28)}$, $371_{(11)}$, $372_{(3)}$, M^+ $386_{(4)}$
II	$C_{25}H_{42}N_2O$	235—237	+96,1	3310, 3150 (OH, NH), 2930 (CH_2-CH_3); 3042, 1463 (methylenecyclopropane ring), 1650, 910 (ring CH_2)	4,75; 4,52 (d, 2H, ring CH_2 CH_2; J 1 Hz 4,03 (m, 1H, CH—OH); 2,43; 2,38 (C 6H, 2N—CH_3); 1,05; 0,91 (s, 6H, CH_3); 1,02 (d, 3H, CH_3; J 6 Hz)	$41_{(7)}$, $42_{(6)}$, $44_{(20)}$, $55_{(5)}$, $56_{(3)}$, **$58_{(100)}$**, $59_{(5)}$, $70_{(6)}$, $328_{(11)}$, $329_{(6)}$, $343_{(8)}$, $356_{(29)}$, $357_{(9)}$, $371_{(31)}$, $372_{(11)}$, M^+ $386_{(21)}$
III	$C_{27}H_{46}N_2O$	198—200	+54,1	3155 (NH) absent	4,90; 4,65 (d, 2H, ring CH_2—CH_2; J 6 Hz 4,00 (m, 1H, CH—OH); 2,28; 2,20 [s, 12H 2N $(CH_3)_2$]; 0,92; 0,78 (s, 6H, CH_3); 0,86 (d, 3H, CH_3; J 6 Hz)	$55_{(6)}$, $58_{(14)}$, $71_{(17)}$, **$72_{(100)}$**, $73_{(5)}$, $342_{(3)}$, $359_{(4)}$, $370_{(14)}$, $371_{(4)}$, $372_{(2)}$, $399_{(4)}$, M^+ $414_{(6)}$.
IV	$C_{31}H_{50}N_2O_4$	238—240	0	1730 (O-Acetyl); 1645 (N-Acetyl)	4,95 (m 1H, HC—OAe); 4,53; 4,43 (d, 2H, ring CH_2—CH_2, J 6 Hz; 1,93 (s, 3H, O—Au); 2,09; 2,00 (C, 6H, 2N—Ac); 1,21; 1,06 (c, 6H, CH_3); 1,03 (d, 3H, CH_3; J 6 Hz)	$55_{(13)}$, $58_{(36)}$, $73_{(4)}$, $74_{(19)}$, **$100_{(100)}$**, $125_{(5)}$, $139_{(8)}$, $290_{(5)}$, $352_{(12)}$, $353_{(18)}$, $354_{(22)}$, $364_{(22)}$, $369_{(13)}$, $378_{(14)}$, $379_{(36)}$, $380_{(22)}$, $439_{(22)}$, $440_{(76)}$, $441_{(36)}$, $453_{(48)}$, $454_{(35)}$, $470_{(24)}$, $497_{(22)}$, $498_{(25)}$, $500_{(14)}$, M^+ $512_{(57)}$
V	$C_{27}H_{46}N_2O$	198—200	+97,7	3150 (NH) absent	4,90; 4,59 (d, 2H, ring CH_2 CH_2; J 1 Hz 3,87 (m, 1H, CH—OH); 2,28; 2,20 [s, 12H, 2N $(CH_3)_2$] 0,92; 0,78 (c, 6H, CH_3) 0,86 (d, 3H, CH_3; J 6 Hz)	$55_{(1)}$, $58_{(16)}$, $71_{(10)}$, **$72_{(100)}$**, $73_{(5)}$, $342_{(13)}$, $359_{(9)}$, $370_{(65)}$, $371_{(69)}$, $372_{(6)}$, $399_{(20)}$, $400_{(6)}$, $412_{(5)}$, **M^+ $414_{(33)}$**
VI	$C_{31}H_{48}N_2O_4$	249—251	0	1740 (O-Acetyl); 1660 (N-Acetyl)	4,93 (m, 1H, HC—OAc); 4,50; 4,35 (d, 2H, ring CH_2—CH_2, J=6 Hz; 1,89 (s, 3H, O—Au); 2,05; 1,96 (c, 6H, 2N—Ac); 1,17; 1,03 (c, 6H, CH_3); 1,07 (d, 3H, CH_3; J=6 Hz)	$55_{(3)}$, $58_{(15)}$, $73_{(12)}$, $74_{(3)}$, **$100_{(100)}$**, $125_{(5)}$, $139_{(11)}$, $290_{(8)}$, $440_{(11)}$, $450_{(6)}$, $451_{(5)}$, $498_{(2)}$, $500_{(1)}$, M^+ $512_{(27)}$.

Note: s — singlet; d — doublet; m — multiplet.

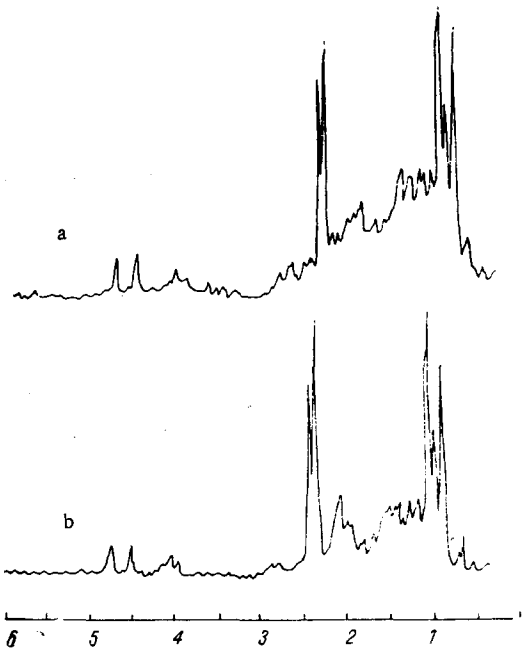


Fig. 2. NMR spectrum of cyclobuxin-D (a) and of pseudocyclobuxin-D (b).

the formation of a N,N',O-triacetyl derivative in the acetylation of (I). In the mass spectrum of (IV) the maximum peak is that of an ion with m/e 100, which is characteristic for a $CH_3-CH-N-COCH_3$ group at C_{17} of the pregnane nucleus.

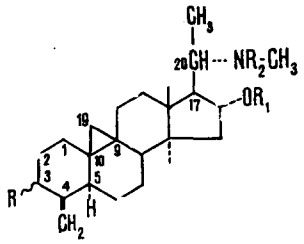
A mixture of (I) and (II) gave no depression of the melting point, the NMR and mass spectra of the two alkaloids and of their derivatives were similar (Fig. 1), and their IR spectra were almost identical (Fig. 2). However, a mixture of the acetyl derivatives of (I) and (II) gave a depression of the melting point. The melting points of the triacetates and the specific rotations of the methylated products of (I) and (II) also differed sharply. In addition, in the mass spectrum of (I) the maximum peak is that of the ion with m/e 44, and in (II) it is that of the ion with m/e 58. The other peaks of the ions in the mass spectra of (I) and (II) also differ in intensity (Table 1).

The ion with m/e 58 from (II) is formed by the cleavage of the $C_{17}-C_{20}$ bond and is due to the $CH_3-CH-NH-CH_3$ fragment, while the ion with m/e 44 is formed by the cleavage of the C_2-C_3 and C_3-C_4 bonds and the migration of hydrogen from position 1. This is explained by the $CH_3-N^+H=CH_2$ ion, amounting to ~20% of the maximum peak [6].

The lower intensity of the peak of the ion with m/e 44 in the spectrum of (II) is due to the presence in it of a 3β-oriented methylamino group [6]. The difference in the intensities of the peaks of the ions in (I) and (II) is apparently connected with a configurational difference in the C_3 asymmetric center, i.e., in (I) the 3-methylamino group is α-oriented. Such cases are possible in the alkaloids of the families Buxaceae, Apocynaceae, and Paxysandrae [9].

The configuration of the other asymmetric centers in (I) follows from the results of a comparison of the values of the chemical shifts of the tertiary and secondary methyl protons with those for cyclobuxin-D (II), i.e., no marked differences in the chemical shifts in (I) and (II) are observed (see Table 1).

Thus, on the basis of what has been said it may be concluded that (I) is an isomer of (II) and has the structure of 14α-methyl-3α,20α-di(methylamino)-4-methylene-9β,19-cyclo-5α-pregnan-16α-ol.



I. R=···NH—CH_3 $R_1=R_2=H$

II. R=—NH—CH_3 $R_1=R_2=H$

III. R=···N(CH_3)(CH_3) $R_1=H$; $R_2=CH_3$

IV. R=···N(CH_3)(Ac) $R_1=R_2=$ Ac

V. R=—N(CH_3)(CH_3) $R_1=H$; $R_2=CH_3$

VI. R=—R(CH_3)(Ac) $R_1=R_2=$ Ac

EXPERIMENTAL

The homogeneity of the substances was checked by chromatography in a thin layer of silica gel in the butan-1-ol–acetic acid–water (10 : 1 : 3) system.

The IR spectra were recorded on a UR-20 spectrometer (tablets with KBr), the NMR spectra (in deuterochloroform) on a JNM-4H-100/100 MHz instrument with HMDS as internal standard (the values are given in the δ scale), and the mass spectra on an MKh-1303 instrument fitted with a glass system for direct introduction into the ion source. The specific rotations of all the compounds were determined in chloroform.

Pseudocyclobuxin-D (I). The leaves and fine flowers of Buxus sempervirens collected on March 25, 1971 in the Shreder experimental station, Tashkent oblast (1 kg) were moistened with 10% ammonia and extracted with chloroform. This gave 14.1 g of combined alkaloids (ethereal fraction 12.15 g, and chloroform fraction 1.95 g).

The ethereal fraction of the combined alkaloids was treated with acetone. Pseudocyclobuxin-D was isolated from the acetone-insoluble fraction; mp 229-231°C (from ethanol), $[\alpha]_D$ +89.64° (c 0.502), R_f 0.15; composition $C_{25}H_{42}N_2O$, mol. wt. 386 (mass spectrometrically).

N,N'-Dimethylpseudocyclobuxin-D (III). A mixture of 0.061 g of pseudocyclobuxin-D, 1 ml of formic acid, and 1 ml of formalin was boiled for 8 h. Then it was diluted with water and made alkaline with ammonia, and the reaction product was extracted with methylene chloride. The residue was treated with ethanol, giving 0.059 g of N,N'-dimethylpseudocyclobuxin-D with mp 198-200°C (from ethanol), $[\alpha]_D$ +54.1° (c 0.61), R_f 0.1, mol. wt. 414 (mass spectrometrically).

N,N',O-Triacetylpseudocyclobuxin-D (IV). A mixture of 0.053 g of (I), 1.3 ml of acetic anhydride, and 1.5 ml of pyridine was kept at room temperature for 46 h. Then the solvent was evaporated off in vacuum, a 3% solution of hydrochloric acid was added, and the reaction product was extracted with methylene chloride. The extract was washed with 3% sodium carbonate solution and then with water. The residue after the distillation of the solvent was treated with acetone–petroleum ether (1 : 9). This gave 0.056 g of N,N',O-triacetylpseudocyclobuxin-D, $C_{31}H_{48}N_2O_4$, mp 238-240°C [acetone–petroleum ether (1 : 9)], $[\alpha]_D$ 0° (c 0.55), R_f 0.70, mol. wt. 512 (mass spectrometrically).

N,N'-Dimethylcyclobuxin-D (V). Cyclobuxin-D (0.2 g) was methylated in a similar manner to (I): the N,N'-dimethylcyclobuxin-D, $C_{27}H_{46}N_2O$, had mp 198-200°C (from ethanol), $[\alpha]_D$ +97.7° (c 0.522), R_f 0.15, mol. wt. 414 (mass spectrometrically).

N,N',O-Triacetylcyclobuxin-D (VI). Cyclobuxin-D (0.22 g) was acetylated by the method described above. The melting point of the N,N',O-triacetylcyclobuxin-D was 249-251°C [from acetone–petroleum ether (1 : 9)], $[\alpha]_D$ 0° (c 0.56); R_f 0.70, mol. wt. 512 (mass spectrometrically).

SUMMARY

1. A new alkaloid, pseudocyclobuxin-D, has been isolated from the leaves and fine flowers of Buxus sempervirens. The results of a comparison of the physicochemical properties of pseudocyclobuxin-D and its derivatives with cyclobuxin-D and its derivatives has shown that the alkaloid isolated is an isomer of cyclobuxin-D and has the structure of 14α-methyl-3α,20α-di(methylamino)-4-methylene-9β,19-cyclo-5α-pregnan-16α-ol.

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