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ALKALOIDS FROM THE LEAVES OF *BUXUS PAPILOSA*

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ABSTRACT.—A new steroidal alkaloid, cycloxobuxoviricine (**1**), has been isolated from the leaves of *Buxus papilosa* and its structure determined by spectroscopic methods. Isolation and ^{13}C-nmr spectrum of another alkaloid, buxaminol B (**2**), not previously reported from the leaves of this plant is also described.

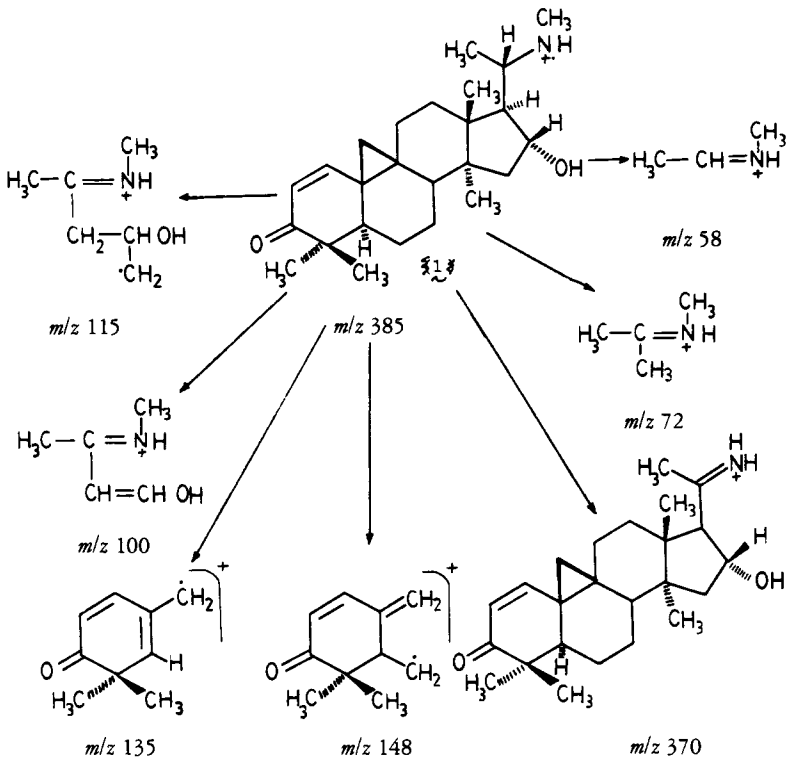
In our continuing studies on the leaves of *Buxus papilosa* C.K. Schnn, Linn. (Buxaceae), we have reported several new alkaloids (1-6). The present investigation describes the isolation and structure elucidation of a new steroidal alkaloid, cycloxobuxoviricine (**1**). Buxaminol B (**2**), which has not previously been reported from the leaves of this plant, was also isolated and its ^{13}C-nmr spectrum recorded.

Cycloxobuxoviricine (**1**) was isolated from the alcoholic extracts of the leaves as a white amorphous solid $[\alpha]^{26}D$ −41.2° ($CHCl_3$). The ir spectrum afforded a strong absorption at 1661 cm^{-1} indicating the presence of an α,β-unsaturated carbonyl group, probably in a six-membered ring. Other absorptions at 3100, 3400, and 3700 cm^{-1} indicated the presence of cyclopropyl, NH, and OH groups, respectively. The uv spectrum showed absorption at 265 nm (log ε 3.59), characteristic of a conjugated ketonic group.

The mass spectrum of **1** showed a molecular ion at *m/z* 385.2955 in agreement with the molecular formula $C_{25}H_{39}NO_2$ (calcd. 385.2980) corresponding to the presence of seven double bond equivalents. The substance readily lost a methyl group to give rise to a peak at *m/z* 370.2728 ($C_{24}H_{36}NO_2$, calcd. 370.2745). The fragments at *m/z* 148.0885 ($C_{10}H_{12}O$, calcd. 148.0888) and *m/z* 135.0808 ($C_9H_{11}O$, calcd. 135.0809) arose by cleavage of ring B (7). Another fragment at *m/z* 115.0982 ($C_6H_{13}NO$, calcd. 115.0997) was formed by the cleavage of ring D along with the nitrogen-bearing side chain, and the presence of an oxygen in this fragment suggested that the OH group was present on ring D, probably attached to C-15, C-16, or C-17. The peak at *m/z* 100.0762, corresponding to the formula $C_5H_{10}NO$ (calcd. 100.0762) formed by the cleavage of ring D, was particularly informative, inasmuch as it established that the HO group was present on a five-carbon fragment. The most probable points of attachment of the HO group were, therefore, at C-16 or C-17. Another important fragment at *m/z* 72.0817 ($C_4H_{10}N$, calcd. 72.0813) arose by cleavage of ring D along with the N-bearing side chain and an intramolecular proton transfer. The absence of oxygen in this four-carbon fragment established that the hydroxyl group was not present on the C-17 carbon atom and pointed to its attachment at C-16.

Compound **1** showed a base peak at *m/z* 58.0655 corresponding to the composition C_3H_8N (calcd. 58.0656), which was attributed to the loss of CH_3-CH=N-CH_3, a commonly encountered fragmentation in other related alkaloids (8). Linked scan measurements of the fragmentation of *m/z* 385 showed that the ions such as *m/z* 370, 148, 135, 115, 100, 72, and 58 directly arose from the molecular ion. The key fragmentations of cycloxobuxoviricine are presented in Scheme 1.

The ^{1}H-nmr spectrum of the cycloxobuxoviricine (**1**) showed four singlets for the four tertiary methyl groups at δ 0.95, δ 0.97, δ 1.10, and δ 1.13, while a doublet centered at δ 1.20 ($J_{21,20}$=6.09 Hz) was assigned to the C-21 secondary methyl group. The presence of a cyclopropyl group was shown by the presence of a doublet at δ 0.76 ($J_{19\alpha,19\beta}$=4.59 Hz). The other doublet was embedded in the methyl/methylenic region of the spectrum. A 3-proton singlet at δ 2.59 was assigned to the N-CH_3 group. A

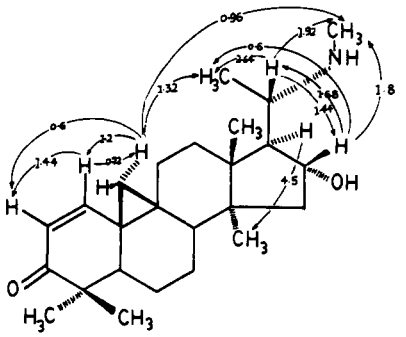


SCHEME 1

double quartet centered at δ 2.98 was assigned to the C-20 proton ($J_{20,21}$=6.09 Hz, $J_{20,17}$=10.63 Hz). A series of homodecoupling experiments was carried out to establish the interrelationship between coupled protons. Irradiation at δ 2.98 resulted in the collapse of the doublet for the 21-methyl group at δ 1.20 into a sharp singlet. The C-17 proton appeared as a double doublet centered at δ 2.13 ($J_{17,16}$=3.93 Hz, $J_{17,20}$=10.63 Hz). Irradiation at δ 2.98 (H-20) also resulted in the collapse of the double doublet for the proton at δ 2.13 into a simple doublet ($J_{17,16}$=3.93 Hz). A multiplet centered at δ 4.28 was assigned to the C-16 proton adjacent to the hydroxyl group. Irradiation at δ 4.28 resulted in the collapse of the double doublet for the C-17 proton into a simple doublet at δ 2.13 ($J_{17,20}$=10.63 Hz). Doublets at δ 5.94 and δ 6.73 ($J_{1,2}$=10.02 Hz) were assigned to C-2 and C-1 olefinic protons, conjugated with the carbonyl group in ring A, respectively.

Two dimensional ^{1}H-nmr measurements (2D-*J* resolved, COSY 45) afforded data that fully agreed with the proposed structure (**1**) for cycloxobuxoviricine. The multiplicity of the proton signals could be unambigously established from the 2-D*J*-resolved spectrum of cycloxobuxoviricine, while the COSY 45 spectrum confirmed the ^{1}H-^{1}H connectivities established by homodecoupling experiments. The nOe difference measurements were also carried out to establish the relative stereochemistry of the various protons. Irradiation of the C-16 proton at δ 4.28 resulted in 1.68% nOe of C-20 H, while irradiation at C-20 H resulted in a corresponding 1.44% nOe of the C-16 proton. This suggested the β-orientation of C-16 H and C-20 H. The absence of any nOe interaction with C-17 H indicated the α-orientation of C-17 H. Irradiation at C-16 H also resulted in 1.8% nOe at N-CH_3 and 0.6% at 21-CH_3. This further supported the β-orientation of C-16 H and, hence, the α-orientation of geminal HO at C-16. Irradiation at C-20 H resulted in 1.92% nOe of N-CH_3 and 2.64% nOe of 18-CH_3. This

showed the β-orientation of C-20 H. Irradiation at C-17 H resulted in 4.5% nOe of 28-CH_3 which established the α-orientation of C-17 H and 28-CH_3. Irradiation at the C-19 α cyclopropyl proton resulted in 1.2% nOe of C-1 olefinic proton, 0.6% nOe of C-2 olefinic proton, 1.32% nOe of 18-CH_3, and 0.96% nOe of N-CH_3, which suggested the relative stereochemistry of the molecule as assigned in structure **1**. This was further supported by NOESY measurements as well as by a comparison of the chemical shifts of protons in cycloxobuxoviricine with other closely related alkaloids having a similar relative stereochemistry (9,10). The ^{13}C-nmr spectrum could not be recorded due to the small quantity of the sample isolated (3.2 mg).



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In the light of the above studies, structure **1** was assigned to cycloxobuxoviricine.

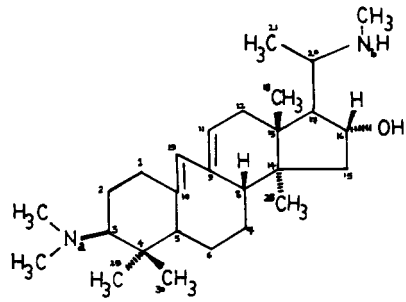
Another alkaloid (**2**), isolated from the leaves, was identified as buxaminol B on the basis of comparison of spectral data (ms, nmr, ir, and uv) reported in the literature (11,12). ^{1}H-nmr assignments were further confirmed by 2-D-^{1}H-nmr experiments (2-D *J*-resolved, COSY 45, NOESY). The ^{13}C-nmr assignments (3,6) of various carbon atoms (Table 1) were substantiated by gated spin echo measurements and agree with the structure (**2**) proposed by Voticky *et al.* (12).

Buxaminol B has not been found previously in the leaves of this plant but has been isolated from *Buxus sempervirens* (12).

TABLE 1. ^{13}C-nmr Spectral Data of Buxaminol B[a]

Carbon No.	Chemical shift (ppm)	Carbon No.	Chemical shift (ppm)
1	37.82	14	47.27
2	30.00	15	43.48
3	71.62	16	77.04
4	41.31	17	62.61
5	49.20	18	17.07
6	23.15	19	127.00
7	25.69	20	58.73
8	51.10	21	15.11
9	138.01	28	18.01
10	137.10	29	24.93
11	128.60	30	15.39
12	32.00	$N_a(CH_3)_2$	44.88
13	44.00	N_bCH_3	32.01

[a]Multiplicities were confirmed by gated spin echo measurements.



2

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were recorded on a Varian MAT 312 double focusing mass spectrometer connected to DEC-PDP 11/34 computer system. The ^{1}H-nmr spectra were recorded in $CDCl_3$ on Bruker AM-300 NMR spectrometer at 300 MHz, while the ^{13}C-nmr spectra were recorded at 75 MHz. Chemical shifts (δ) are expressed in ppm. Ir spectra were recorded on a Jasco IRA-1 infrared spectrometer. The uv spectra were recorded on Shimadzu UV 240 instrument. The optical rotation was determined with a Polartronic D instrument.

PLANT MATERIAL.—The leaves of *B. papilosa* were collected from the northern region of Pakistan by the Forest Institute, Peshawar. The plant was identified by Prof. S. Irtifaq Ali, Department of Botany, University of Karachi, and a voucher specimen has been deposited in the Department of Botany, University of Karachi.

EXTRACTION AND PURIFICATION.—The EtOH extract of the air-dried leaves (10 kg) of *B. papilosa* was evaporated under vacuum to afford a gum (48 g). This gum ws taken up in 10% HOAc. The aqueous acidic mixture was extracted with $CHCl_3$ on the basis of differential basicities.

ISOLATION OF CYCLOXOBUXOVIRICINE (**1**).—The fraction obtained at pH 4.0 (14 g) was loaded on a silica gel column (450 g) that was successively eluted with increasing polarities of mixtures of Me_2CO-MeOH (6:4) contained a number of alkaloids (2.00 g). The mixture was subjected to repeated preparative tlc (silica gel) in hexane-EtOAc-diethylamine (18:3:1), which afforded cyclobuxoviridine, *N*-benzoylbuxidienine, cyclobuxoviricine along with a minor new alkaloid, cycloxobuxoviricine (**1**), as a white amorphous powder (3.2 mg), $[\alpha]^{26}D$ −41.2° $CHCl_3$; ir ν max ($CHCl_3$), 3700, 3400, 3100, 1661, 1600 cm^{-1}; uv λ max (MeOH) 265 nm (log 3.59); ^{1}H nmr (300 MHz, $CDCl_3$) δ 0.76 (d, 1H, $J_{19\alpha,19\beta}$=4.59 Hz, 19α C*H*), 0.95 (s, 3H, t-CH_3), 0.97 (s, 3H, t-CH_3), 1.10 (s, 3H, t-CH_3), 1.13 (s, 3H, t-CH_3), 1.20 (d, 3H, $J_{21,20}$=6.09 Hz, 21-CH_3), 2.13 (dd, 1H, $J_{17,16}$=3.93 Hz, $J_{17,20}$=10.63 Hz, 17-C*H*), 2.59 (s, 3H, N-CH_3), 2.98 (dq, 1H, $J_{20,21}$=6.09 Hz, $J_{20,17}$=10.63 Hz, 20-C*H*), 4.28 (m, 1H, 16-CH_3), 5.94 (d, 1H, $J_{2,1}$=10.02 Hz, 2-C*H*), 6.73 (d, 1H, $J_{1,2}$=10.02 Hz, 1-C*H*); ms *m/z* 385.2980 (M^+, $C_{25}H_{39}NO_2$), 370.2728 ($C_{24}H_{36}NO_2$), 148.0875 ($C_{10}H_{12}O$), 135.0808 ($C_9H_{11}O$), 115.0982 ($C_6H_{13}NO$), 100.0762 ($C_5H_{10}NO$), 72.0817 (C_4H_9N), 58.0655 (C_3H_8N).

ISOLATION OF BUXAMINOL B (**2**).—The defatted aqueous acidic extract was then basified with NH_3 solution (pH 8.5) and extracted with EtOAc (6 liters). The crude alkaloids, obtained on evaporation of the EtOAc extracts (8 g), were subjected to preparative tlc (silica gel) in C_6H_{14}-Et_2NH-Me_2CO (18:1:1) to afford the alkaloid, buxaminol B, (ms, nmr, ir, and uv), as a light yellow amorphous powder (20 mg), [α]D 17.51° $CHCl_3$; ^{13}C nmr (75 MHz, $CDCl_3$, δ), Table 1; *m/z* 414.359 (M^+, $C_{27}H_{46}N_2O$).

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