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NEW ALKALOIDS FROM *BUXUS PAPILOSA*[1]

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ABSTRACT.—*Buxus papilosa* C.K. Schneider (Buxaceae) of Pakistani origin has yielded the new steroidal alkaloids (+)-buxabenzamidine [**1**], (+)-homobuxaquamarine [**2**], (+)-norcyclomicrobuxeine [**3**], (+)-buxupapine [**4**], and (+)-N_b-norbuxupapine [**5**]. The known base cyclobuxoviridine [**6**] was also reisolated. It is levorotatory, and some details of its nmr spectrum have been clarified.

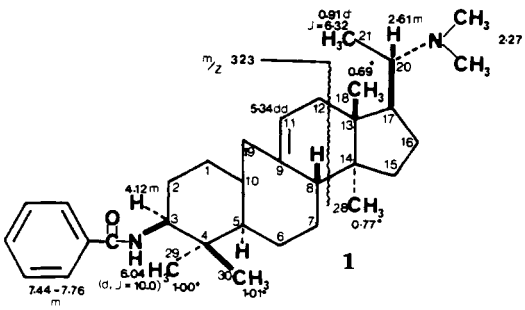
Buxus papilosa C.K. Schneider (Buxaceae) is a shrub growing extensively in the northern regions of Pakistan. *Buxus* species extracts have been used in the indigenous system of medicine for a variety of purposes, including as a febrifuge and for the relief of rheumatism and malaria (1).

Previous studies of *Buxus* species have resulted in the isolation of more than 100 steroidal alkaloids. All of these compounds possess either the pentacyclic cyclo-9β,19-pregnane-5α, or the tetracyclic *abeo*-9(10→19)-pregnane-5α skeleton, with minor structural variations possible.

We have already reported on 18 new alkaloids as a result of our continuing studies on the leaves of *B. papilosa* (2-12). Presently, we describe the isolation and structure elucidation of five new steroidal bases, (+)-buxabenzamidine [**1**], (+)-homobuxaquamarine [**2**], (+)-norcyclomicrobuxeine [**3**], (+)-buxupapine [**4**], and (+)-N_b-norbuxupapine [**5**]. The known base (−)-cyclobuxoviridine [**6**], which had previously been found in *Buxus microphylla*, was also obtained (13). All of these compounds have been isolated from the so-called weakly basic alkaloidal fractions of *B. papilosa* leaves extractable between pH 3.5 and 5.0.

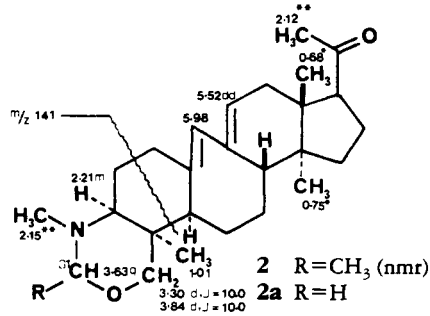
(+)-Buxabenzamidine [**1**], $C_{33}H_{50}N_2O$, shows a uv spectrum with a maximum at 226 nm, characteristic of a benzamidic chromophore (14). The ir spectrum in $CHCl_3$ solution displays intense bands at 3670 (N-H) and 1650 cm^{-1} (aromatic amide).

The 360 MHz ($CHCl_3$) nmr spectrum of (+)-buxabenzamidine, summarized around expression **1**, featured four singlets for the four tertiary methyl groups at δ 0.69, 0.77, 1.00, and 1.01; while a doublet centered at δ 0.91 was assigned to the C-



1

H-3α, J_1=12.5 Hz, J_2=10.0 Hz, J_3=3.0 Hz
H-11, J_1=2.1 Hz, J_2=1.6 Hz



2 R=CH_3 (nmr)
2a R=H

C-31 CH_3, J=5.4 Hz (d, δ 1.33)
H-31, J=5.4 Hz
H-11, J_1=2.5 Hz, J_2=1.6 Hz

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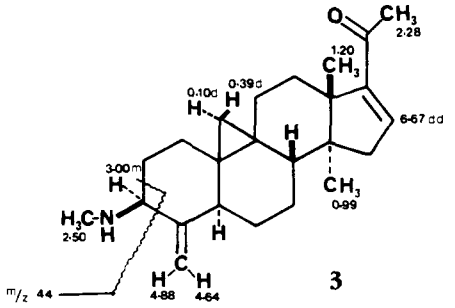
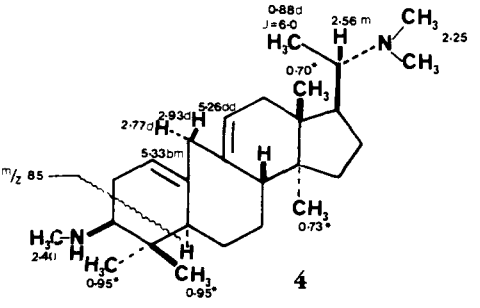


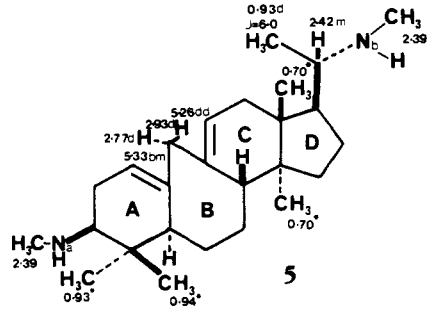
3

H-3α, J_1=11.7 Hz, J_2=4.1 Hz
H-16, J_1=3.4 Hz, J_2=2.0 Hz
H-19α, J=4.4 Hz; H-19β, J=4.4 Hz



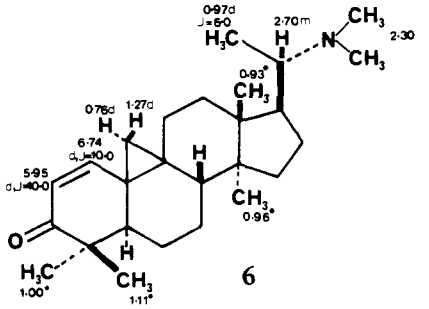
4

H-11, J_1=2.5 Hz, J_2=1.5 Hz
H-19α, J=12.0 Hz; H-19β, J=12.0 Hz



5

H-11, J_1=2.5 Hz, J_2=1.4 Hz
H-19α, J=12.0 Hz; H-19β, J=12.0 Hz



6

H-19α, J=4.6 Hz; H-19β, J=4.6 Hz

*Chemical shifts with identical superscripts are interchangeable.

21 secondary methyl group. A six-proton singlet at δ 2.27 was ascribed to the *N*-methyl groups. A multiplet centered at δ 4.12 represented H-3α. A doublet of doublets at δ 5.34 was related to the vinylic H-11, whose signal is split by the C-12 methylene protons (15).

The mass spectrum of (+)-buxabenzamidine [**1**] includes molecular ion *m/z* 490. Peak *m/z* 323 is the result of retro-Diels-Alder cleavage of ring C. Base peak *m/z* 72 represents the trimethyliminium cation, $H_3C\text{-}CH{=}N(CH_3)_2^+$.

Our second compound, (+)-homobuxaquamarine [**2**], $C_{27}H_{41}NO_2$, showed a uv spectrum with maxima at 236 and 245 nm, and shoulders at 225 and 255 nm, characteristic of 9(10↦19)*abeo*diene bases. Similar absorptions have been encountered in the cases of (+)-moenjodaramine (4), (+)-harappamine (4), and (+)-buxaquamarine (9). The ir spectrum displayed a strong absorption at 1695 cm^{-1} indicating the presence of a carbonyl group.

The ^{1}H-nmr spectrum of **2** bore a distinct similarity to that of (+)-buxaquamarine [**2a**] (9). It incorporated signals due to three tertiary methyl groups at δ 0.68, 0.75, and 1.01. A three-proton singlet at δ 2.12 was assigned to the methyl group adjacent to the carbonyl function. Another three-proton singlet at δ 2.15 could be assigned to the *N*-methyl group. A set of AB doublets resonating at δ 3.30 and 3.84 represented the C-30 methylene protons. However, unlike the spectrum of (+)-buxaquamarine [**2a**], the C-31 proton appeared as a quartet at δ 3.63. Additionally, a three-proton doublet at δ 1.33 indicated that this methyl group was attached to C-31 in the tetrahydrooxazine ring. The H-11 allylic proton appeared as a doublet of doublets at δ 5.52, while H-19 was in the form of a singlet at δ 5.98.

The mass spectrum of (+)-homobuxaquamarine [**2**] displayed molecular ion *m/z*

411. Base peak *m/z* 396 corresponded to the loss of a methyl group. Another peak *m/z* 368 resulted from loss of acetyl. An important fragment *m/z* 141 could arise by cleavage of ring A as indicated.

The third alkaloid, (+)-norcyclomicrobuxeine [**3**], $C_{24}H_{35}NO$, manifests a uv spectrum with a maximum at 240 nm, indicating the presence of an α,β-unsaturated ketone (13). The ir spectrum incorporates bands at 3680 (N-H), 1657 (conjugated carbonyl), and 1588 cm^{-1} (C=C).

The ^{1}H-nmr spectrum of (+)-norcyclomicrobuxeine [**3**] displayed AB doublets at δ 0.10 and 0.39 accounting for the cyclopropyl methylenic protons. Signals due to two tertiary methyl groups were at δ 0.99 and 1.20. A three-proton singlet at δ 2.28 was assigned to a methyl group next to the carbonyl. Another three-proton singlet at δ 2.50 represented the *N*-methyl group. A multiplet at δ 3.00 was related to H-3α. The terminal methylidene protons appeared as singlets at δ 4.64 and 4.88. The C-16 olefinic proton absorbed as a doublet of doublets at δ 6.67. The overall spectral image thus resembled that for (+)-cyclomicrobuxeine (13), and in fact species **3** corresponds to the *N*-nor derivative of (+)-cyclomicrobuxeine.

Some nmr-nOe difference measurements were obtained on **3** in order to clarify specific structural features and confirm some of the assignments. Irradiation of the C-21 methyl group (δ 2.28) led to an 11.1% enhancement of H-16 (δ 6.67), while irradiation of H-16 resulted in a 7.8% increase of the C-21 methyl signal. These data supported a transoid geometry for the enone system. Similarly, reciprocating nOe's were found between the exocyclic vinylic proton signal at δ 4.88 and the *N*-methyl at 2.50.

The mass spectrum of the compound includes molecular ion *m/z* 353. Ion *m/z* 310 results from loss of the acetyl group. Base peak *m/z* 44 derives from cleavage of ring A as indicated with proton transfer.

(+)-Buxupapine [**4**], $C_{27}H_{46}N_2$, is the fourth new alkaloid obtained. Its uv spectrum shows only terminal absorption, and the ir spectrum includes bands at 3670 (N-H) and 1600 cm^{-1} (C=C).

The ^{1}H-nmr spectrum of (+)-buxupapine [**4**] includes four singlets for the four tertiary methyl groups at δ 0.70, 0.73, 0.95, and 0.95. The C-21 secondary methyl group is represented by a doublet at δ 0.88. A six-proton singlet at δ 2.25 is diagnostic of a dimethylamino group and another singlet at δ 2.40 of an *N*-methyl. A doublet of doublets centered at δ 5.26 can be ascribed to H-11 which is split by the C-12 methylene protons. Another broad multiplet at δ 5.33 is due to H-1. The two double bonds are not conjugated, and this finding is in accord with the lack of a distinct uv absorption.

The mass spectrum of (+)-buxupapine [**4**] exhibits molecular ion *m/z* 398. Peak *m/z* 355 reflects the loss of $HC{=}NHCH_3^+$ from ring A. Peak *m/z* 85 results from retro-Diels-Alder cleavage of ring A (16). Base peak *m/z* 72 represents the trimethylimimium side chain.

(+)-Buxupapine [**4**] is accompanied in the plant by its *N*-demethyl analog, (+)-N_b-norbuxupapine [**5**], $C_{26}H_{44}N_2$. Compound **5** has an ir spectrum very close to that of **4**, and its uv spectrum again shows only terminal absorption.

The ^{1}H-nmr spectrum of (+)-N_b-norbuxupapine [**5**] has been delineated around expression **5** and is very close to that of (+)-buxupapine [**4**]. The one glaring difference is that two *N*-methyl group absorptions are present, instead of three.

The mass spectrum of (+)-N_b-norbuxupapine [**5**] includes molecular ion *m/z* 384. Peak *m/z* 341 is due to loss of $CH{=}NHCH_3^+$ from ring A. Peak *m/z* 85 results from retro-Diels-Alder cleavage of ring A. Finally, base peak *m/z* 58 is characteristic of the dimethyliminium cation, $H_3C\text{-}CH{=}NHCH_3^+$, produced by fission of the ring D side chain.

Our sixth alkaloid was identified as (−)-cyclobuxoviridine [**6**] by comparison of its spectral data (ms, nmr, uv, and ir) with those reported in the literature (13). Presently, ^{1}H-nmr spectral assignments were confirmed and extended by a series of homodecoupling experiments. In particular, H-19β and H-19α appear as AB doublets at δ 1.27 and 0.76, respectively. The downfield shift from the usual values (17) is due to the deshielding effect of the neighboring olefinic functionality.

Cyclobuxoviridine [**6**] had previously been found in *B. microphylla*. Its specific rotation had been recorded as being +16° (*c* 0.40, $CHCl_3$). Our optical value for this alkaloid was −20° (*c* 1.38, $CHCl_3$. Because other *Buxus* alkaloids closely related to cyclobuxoviridine and possessing a C-1,2 double bond such as cyclobuxoviricine (10) and cycloxobuxoviricine (11) possess negative specific rotations, it is conceivable that the sign for the specific rotation originally recorded for cyclobuxoviridine [**6**] should be changed.

EXPERIMENTAL

All ^{1}H-nmr spectra are at 360 MHz in $CDCl_3$ solution. Molecular compositions were obtained using low resolution ms.

PLANT MATERIAL.—The leaves of *B. papilosa* (dry weight 50 kg) were collected in northern Pakistan, in January 1984, by the Forest Institute, Peshawar. The plant was identified by Prof. S. Irtifaq Ali, Department of Botany, University of Karachi, and a voucher specimen was deposited in the herbarium of the Department of Botany, University of Karachi.

EXTRACTION AND PURIFICATION.—Extraction was with EtOH at room temperature. The solvent was evaporated in vacuo to afford a gum (110 g) which was taken up in 10% HOAc. The pH was then adjusted by addition of 20% HOAc.

ISOLATION OF (+)-BUXABENZAMIDINE [**1**], (+)-HOMOBUXAQUAMARINE [**2**], (+)-NORCYCLOMICROBUXEINE [**3**], AND (−)-CYCLOBUXOVIRIDINE [**6**].—The fraction obtained by extraction with $CHCl_3$ at pH 3.5 was loaded on a silica gel column (70-230 mesh, 250 g). The initial solvent was $CHCl_3$. Elution was with $CHCl_3$ and then with $CHCl_3$ gradually enriched with MeOH. Four main fractions were obtained: Fraction A, $CHCl_3$-MeOH (95:5), 1.5 g; Fraction B, $CHCl_3$-MeOH (92:8), 1.0 g; Fraction C, $CHCl_3$-MeOH (92:8), 1.4 g; Fraction D, $CHCl_3$-MeOH (90:10), 1.7 g.

(+)-BUXABENZAMIDINE [**1**].—Fraction C was placed on a silica gel column (70 g). Elution was with $CHCl_3$-MeOH-NH_4OH (98:8:1). An important fraction (18 mg) was subjected to repeated preparative tlc (silica gel) in the solvent system C_6H_6-$(C_2H_5)_2NH$ (96:4) to supply **1** (6 mg), amorphous, [α]D +47° (*c* 0.73, $CHCl_3$); ir ν max ($CHCl_3$) 3670, 1650, 1600 cm^{-1}; uv λ max 226 nm (log ε 4.89); ms *m/z* 490 (M^+, 1), 475 (1), 323 (1), 322 (2), 105 (8), 73 (7), 72 (100), 58 (2), 44 (2).

(+)-HOMOBUXAQUAMARINE [**2**].—Fraction D was further chromatographed over a silica gel column. Elution was with $CHCl_3$-MeOH-NH_4OH (90:10:1). An important fraction (12 mg) was purified by tlc using the system C_6H_6-$(C_2H_5)_2NH$ (96:4) to supply amorphous **2** (1.4 mg), [α]D +21.9° (*c* 0.64, $CHCl_3$); ir ν max ($CHCl_3$) 1695, 1645, 1206 cm^{-1}; uv λ max (MeOH) 225 sh, 236, 245, 255 sh nm (log ε 4.15, 4.26, 4.31, 4.13); ms *m/z* 411 (M^+, 45), 396 (100), 368 (4), 141 (14), 98 (13), 85 (41), 72 (23), 58 (58), 57 (20), 44 (20), 43 (56).

(+)-NORCYCLOMICROBUXEINE [**3**].—Fraction A was placed on a silica gel column (85 g). Elution was with C_6H_{14}-$CHCl_3$-$(C_2H_5)_2NH$ (80:15:5). Further purification was by tlc to supply amorphous **3** (3 mg), [α]D +34° (*c* 1.90, $CHCl_3$); ir ν max ($CHCl_3$) 3680, 1657, 1588 cm^{-1}; uv λ max (MeOH) 240 nm (log ε 3.89); ms *m/z* 353 (M^+, 21), 337 (13), 310 (7), 44 (100).

(−)-CYCLOBUXOVIRIDINE [**6**].—Fraction B was loaded on a silica gel column (70 g). Elution was with $CHCl_3$-MeOH-NH_4OH (96:4:0.4). An important fraction was then purified further by tlc to afford **6** (4 mg), light yellow amorphous powder, [α]D −20° (*c* 1.38, $CHCl_3$); ir ν max ($CHCl_3$) 1662, 1600 cm^{-1}; uv λ max (MeOH) 267 nm (log ε 3.92); ms *m/z* 383 (M^+, 1), 368 (2), 84 (3), 72 (100), 58 (7), 44 (4).

ISOLATION OF (+)-BUXUPAPINE [**4**] AND (+)-N_b-NORBUXUPAPINE [**5**].—The alkaloidal fraction obtained by extraction with $CHCl_3$ at pH 5 (2 g) was loaded on a silica gel column (70 g), and eluted with C_6H_{14}-$CHCl_3$-MeOH-$(C_2H_5)_2NH$ (75:15:5:5). The important fraction was then purified by tlc to supply amorphous **4** (3 mg) and **5** (3 mg).

(+)-BUXUPAPINE [**4**].—[α]D +11° (*c* 1.13, $CHCl_3$); ir ν max ($CHCl_3$) 3670, 1600 cm^{-1}; ms *m/z* 398 (M^+, 2), 383 (2), 355 (2), 85 (7), 73 (5), 72 (100), 58 (2), 44 (3), 43 (2).

(+)-N_b-NORBUXUPAPINE [**5**].—[α]D +20° (*c* 0.71, $CHCl_3$); ir ν max ($CHCl_3$) 3650, 1600 cm^{-1}; ms *m/z* 384 (M^+, 4), 341 (7), 300 (4), 85 (37), 72 (33), 58 (100), 43 (6).

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