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FOUR STEROIDAL ALKALOIDS FROM *BUXUS PAPILOSA*

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Key Word Index—*Buxus papilosa*; Buxaceae; steroidal alkaloids; (+)-buxaprogestine; (−)-buxapapinolamine; (−)-*Z*-cyclobuxaphylamine; (−)-*E*-cyclobuxaphylamine; (+)-*N*-benzoylcycloxobuxine-F.

Abstract—Four new steroidal alkaloids from *Buxus papilosa* of Pakistani origin are (+)-buxaprogestine, (−)-buxapapinolamine, (−)-*E*-cyclobuxaphylamine and (−)-*Z*-cyclobuxaphylamine. Some features of the ^{1}H NMR spectrum of the known base (+)-*N*-benzoylcycloxobuxine-F are also clarified.

INTRODUCTION

Buxus papilosa C. K. Schneider (Buxaceae) is an evergreen shrub which is found in the northern regions of Pakistan. Water extracts of this plant have found extensive use in the indigenous system of medicine [1]. Previous studies on the ethanolic extracts of *B. papilosa* leaves resulted in the isolation of 23 new alkaloids [2–14]. These incorporate either the pentacyclic 9β,19-cyclo-5α-pregnane or the tetracyclic 9(10 → 19)*abeo*-5α-pregnane systems. Presently, we describe the isolation and structure elucidation of four new steroidal bases, (+)-buxaprogestine (**1**), (−)-buxapapinolamine (**2**), (−)-*E*-cyclobuxaphylamine (**3**) and (−)-*Z*-cyclobuxaphylamine (**4**). The known base (+)-*N*-benzoylcycloxobuxine-F (**5**), not previously reported in this plant is also described, and some of the features of its ^{1}H NMR spectrum are clarified.

RESULTS AND DISCUSSION

The compounds were isolated from the so-called weakly basic alkaloidal fractions of *B. papilosa* leaves, extracted at a pH between 3.5 and 5.0. (+)-Buxaprogestine (**1**), $C_{23}H_{37}NO$, shows a UV spectrum with a maximum at 239 nm, characteristic of an α,β-unsaturated ketone. The IR spectrum displayed bands at 1660 (C=C–C=O) and 1610 (C=C) cm^{-1}.

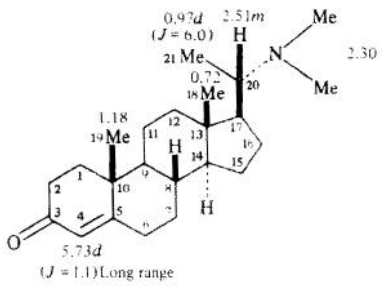
The ^{1}H NMR spectrum ($CDCl_3$, 360 MHz) included two singlets at δ0.72 and 1.18, corresponding to the C-18 and C-19 methyl groups, respectively. The secondary C-21 methyl group resonated as a doublet at δ0.97, while the neighbouring C-20 methine proton appeared as a multiplet at δ2.51. A six-proton singlet at δ2.30 was assigned to the dimethylamino function. A close doublet centered at δ5.73 can be ascribed to the C-4 olefinic proton which is coupled with H-6. The mass spectrum of (+)-buxaprogestine (**1**) features molecular ion *m/z* 343. Base peak *m/z* 72 represents the trimethyliminium cation formed through cleavage of the C-17 to C-20 bond. Other important peaks are *m/z* 328, 84 and 58. (+)-Buxaprogestine (**1**) represents only the second example of the occurrence of a simple pregnane derivative within the Buxaceae. The first example, (−)-irehine, was found in *B. sempervirens* [15].

Our second alkaloid, (−)-buxapapinolamine (**2**), $C_{35}H_{48}N_2O_5$, possessed a UV spectrum with a maximum at 227 nm, characteristic of a benzamidic chromophore [16]. The IR spectrum (in chloroform) displayed intense bands at 3670 (N-H), 3310 (O-H), 1732 (COOR), 1722 (CHO) and 1658 (aromatic amide) cm^{-1}.

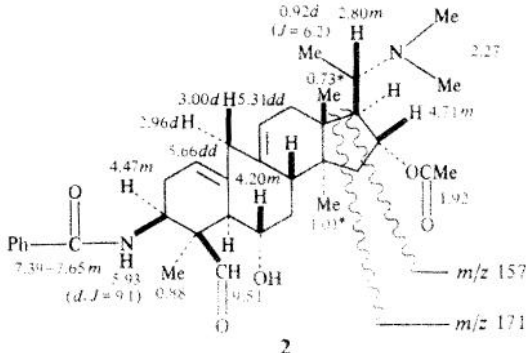
The NMR spectrum of **2** manifested signals representing three tertiary methyl groups at δ0.73, 0.88 and 1.01. A three-proton doublet at δ0.92 was assigned to the secondary methyl group of the side chain. Another singlet relatively downfield at δ1.92 was diagnostic of an acetate methyl group. The dimethylamino function appeared as a singlet at δ2.27. A multiplet at δ4.71 was due to H-16 which is geminal to the acetate function. A doublet of doublets at δ5.31 and another at δ5.66 represented the vinylic hydrogens at C-11 and C-1, respectively. The two double bonds in (−)-buxapapinolamine (**2**) are thus not conjugated, and this finding is in accord with the UV absorption. The aldehydic proton absorbed as a singlet at δ9.51. An interesting feature of the NMR spectrum was the presence of a multiplet at δ4.20 denoting H-6 which is geminal to the hydroxyl group.

In accord with all other related *Buxus* alkaloids, the C-3 aminated substituent in (−)-buxapapinolamine (**2**) has been placed in a beta configuration. Furthermore, whenever biogenetic oxidation of the *gem*-dimethyl substituent of ring A obtains, it is always the 4β-methyl (C-31) that is affected. It is relevant to point out that more than a dozen 6α-hydroxycycloartenol triterpenoids are known which possess an alcohol function in ring B at exactly the same site as in (−)-buxapapinolamine (**2**) [17].

The NMR spectrum of species **2** was also rerun in pyridine-d_5 [18]. It is known that under these conditions protons adjacent to the hydroxyl group will suffer a pronounced paramagnetic shifts in relation to the $CDCl_3$ spectrum. Indeed, the downfield shifts experienced by the C-31 aldehydic proton (δ9.51–9.94), the C-6 proton (δ4.20–4.91) and the C-30 methyl protons (δ0.88–1.24) argue convincingly in favour of the proposed C-6α position for the hydroxyl function [17, 18].

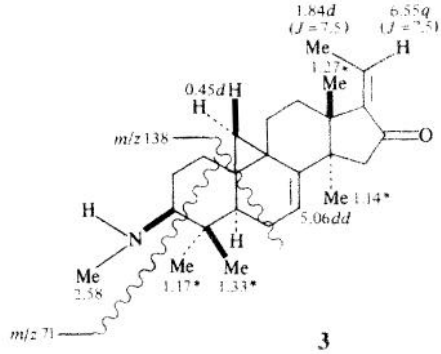


1



2

H-1, J_1 = 6.0 Hz, J_2 = 2.0 Hz
H-19α, J = 10.0 Hz, H-19β, J = 10.0 Hz
H-11, J_1 = 2.0 Hz, J_2 = 1.0 Hz



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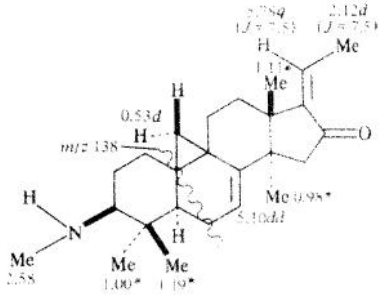
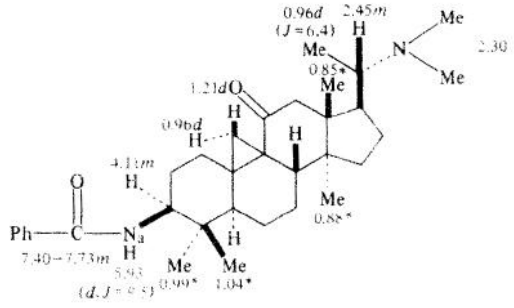


4

H-6, J_1 = 5.4 Hz, J_2 = 2.0 Hz
H-19α, J = 4.4 Hz

H-6, J_1 = 5.4 Hz, J_2 = 2.4 Hz
H-19α, J = 4.0 Hz



5

H-19α, J = 4.6 Hz, H-19β, J = 4.6 Hz

The mass spectrum of **2** evidenced molecular ion *m/z* 576. Loss of the trimethyliminium side chain accounted for base peak *m/z* 72. Other significant ions were *m/z* 157 and 171 which are formed by cleavage of ring D along the lines indicated. It follows that the acetoxyl group must be attached to ring D. More specifically, this function is linked to C-16, since it is known that the 16α-acetoxyl substituent is present in a variety of *Buxus* alkaloids [16]. Finally, peak *m/z* 105 is due to the benzoyl substituent, while the relatively large *m/z* 28 peak may be accounted for through loss of carbon monoxide. Acetylation of (−)-buxapapinolamine (**2**) using acetic anhydride in pyridine afforded as expected (−)-6α-acetylbuxapapinolamine, $C_{37}H_{50}N_2O_6$. The NMR spectrum of this acetate (Experimental) showed a downfield shift of H-6β from δ4.20 to 5.18 and a new singlet at δ1.90 representing the acetyl protons. Alternatively, sodium borohydride in methanol reduction of (−)-buxapapinolamine (**2**) supplied the corresponding C-31 alcohol, $C_{35}H_{50}N_2O_5$, whose IR spectrum showed the lack of aldehydic absorption near 1722 cm^{-1}. The NMR spectrum (Experimental) included two one proton doublets at δ3.68 and 3.78 representing the C-31 methylene hydrogens.

Our two remaining new bases are *E*- and *Z*-

cyclobuxaphylamine. (−)-*E*-cyclobuxaphylamine (**3**), $C_{25}H_{37}NO$, has a UV absorption with a maximum at 244 nm, characteristic of an α,β-unsaturated ketone. The IR spectrum displayed peaks at 1712 and 1636 (α,β-unsaturated cyclopentanone) [19].

The NMR spectrum of (−)-*E*-cyclobuxaphylamine (**3**) featured four singlets at δ1.14, 1.17, 1.27 and 1.33, corresponding to the four tertiary methyl groups. Only half of the cyclopropyl AB quartet could be observed at δ0.45, while the other half had shifted to the methylmethylene region due to the deshielding influence of the neighboring olefinic functionality. A doublet at δ1.84 was due to the 21-methyl group which is coupled with H-20. H-20 in turn appeared as a quartet at δ6.55 [19, 20]. A singlet at δ2.58 was readily assigned to the *N*-methyl residue. The H-7 vinylic proton resonated as a doublet of doublets at δ5.06 [21].

The mass spectrum of (−)-*E*-cyclobuxaphylamine was particularly informative. It included molecular ion *m/z* 367. An *m/z* 138 peak was the result of the allylic cleavage of ring B along the line indicated. This ion was important in locating the position of the double bond at C-7(8). Base peak *m/z* 71 resulted from ring A cleavage as shown in expression **3**.

(−)-*E*-Cyclobuxaphylamine (**3**) was accompanied by its geometric isomer, (−)-*Z*-cyclobuxaphylamine (**4**), $C_{25}H_{37}NO$. Compound **4** shows a UV spectrum with a maximum at 240 nm. The IR spectrum includes peaks at 1709 and 1637 (α,β-unsaturated cyclopentanone).

The NMR spectrum of (−)-*Z*-cyclobuxaphylamine (**4**) resembled that of **3**, except that the C-20 vinylic proton fell relatively upfield at δ5.78. In contrast, the 21-methyl doublet at δ2.12 was further downfield than in the case of **3** since this methyl is now *syn* to the C-16 carbonyl oxygen [20]. The mass spectrum exhibited molecular ion *m/z* 367, while the base peak *m/z* 71 was again due to cleavage of ring A. Another important peak was *m/z* 138, resulting from fission of ring B along the lines indicated. It has been justifiably pointed out in the literature that species of types **3** and **4** could be true alkaloids, or else could be artifacts of isolation produced through β-elimination of the corresponding C-20 dimethylamino analogue [19].

Our fifth compound was identified as the known (+)-*N*-benzoylcycloxobuxine-F(**5**) by comparison of its spectral properties with those reported in the literature [16]. Presently, however, NMR spectral assignments were confirmed and extended through a series of homodecoupling experiments. In particular, H-19β and H-19α appear as AB doublets at δ1.21 and 0.96, respectively. The downfield shift from the usual values [22] is due to the deshielding effect of the neighboring carbonyl group. Compound **5** had not been found previously in the leaves of *B. papilosa*, but had been isolated from *B. sempervirens* [16].

The signs of the specific rotations for the above mentioned alkaloids are in accord with the rules relating specific rotations to structural features for the *Buxus* alkaloids [13].

EXPERIMENTAL

All ^{1}H NMR were recorded at 360 MHz in $CDCl_3$ solution.

The leaves of *B. papilosa* (dry 50 kg) were collected in the northern regions of 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. The EtOH extract of *B. papilosa* leaves was evaporated to a gum. Total alkaloids (110 g) were obtained by extraction into 10% HOAc. Partial separation of the alkaloids was carried out by extraction into $CHCl_3$ at different pH values. The fraction obtained at pH 3.5–5.0 (10 g) was loaded on a silica gel column (250 g). Elution was with $CHCl_3$ and then with $CHCl_3$–MeOH. Four main fractions were obtained: Fraction A, $CHCl_3$–MeOH (19:1), 0.54 g; Fraction B, $CHCl_3$–MeOH (95:8), 0.57 g; Fraction C, $CHCl_3$–MeOH (23:2), 1.4 g; Fraction D, $CHCl_3$–MeOH (9:1), 1.7 g.

(+)-*Buxaprogestine* (**1**). Fraction B was placed on a silica gel column (38 g). Elution was with C_6H_{14}–Et_2NH (19:1). An important fraction (15.0 mg) was subjected to repeated TLC (silica gel) in the solvent system C_6H_6–Et_2NH (98:2) to supply **1** (1.9 mg), amorphous, $[\alpha]_D = +26$ ($CHCl_3$; *c* 1.78). UV λ_{max}^{MeOH} nm: 239 (log ε 4.20); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1660, 1610; MS *m/z* (rel. int.): 343 [M, 1]$^+$, 328 (2), 112 (1), 84 (10), 72 (100), 58 (12).

(−)-*Buxapapinolamine* (**2**). Fraction D was placed on a silica gel column (100 g) and eluted with $CHCl_3$–MeOH–NH_4OH (90:10:1). An important fraction (8.0 mg) was purified by TLC (silica gel) using the solvent system C_6H_6–Et_2NH (19:1) to supply amorphous **2** (4.3 mg), $[\alpha]_D = -16$ ($CHCl_3$; *c* 1.84). UV λ_{max}^{MeOH} nm: 227 (log ε 4.00); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3670, 3310, 1732, 1722, 1658; MS *m/z* (rel. int.): 576 [M, 1]$^+$, 561 (2), 171 (2), 157 (2), 105 (5), 84 (2), 72 (100), 60 (2), 58 (4), 44 (2), 28 (4).

Acetylation of (−)-*Buxapapinolamine* (**2**). (−)-Buxapapinolamine (**2**) was acetylated (Ac_2O/pyr.) at room temperature. The product exhibited, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 1732, 1722, 1660; ^{1}H NMR: δ0.74 (3H, *s*, *t*-Me), 0.88 (3H, *s*, *t*-Me), 0.91 (3H, *d*, *J* = 6.4 Hz, 21-Me), 1.00 (3H, *s*, *t*-Me), 1.90 (3H, *s*, COMe), 1.98 (3H, *s*, COMe), 2.26 (6H, *s*, $N(Me)_2$), 4.45 (1H, *m*, 3-H), 4.70 (1H, *m*, 16-H), 5.18 (1H, *m*, 6-H), 5.35 (1H, *dd*, 11-H), 5.68 (1H, *m*, 1-H), 5.93 (1H, *d*, J = 9.0 Hz, NH), 7.36–7.66 (5H, 2 *m*, ArCOH), 9.47 (1H, *s*, COH); MS *m/z* (rel. int.): 618 [M, 1]$^+$, 603 (1), 575 (2), 559 (1), 558 (2), 171 (1), 157 (1), 105 (13), 72 (100), 60 (2), 58 (3), 43 (4), 28 (6).

Reduction of (−)-*Buxapapinolamine* (**2**). Reduction of **2** with sodium borohydride in MeOH supplied the corresponding alcohol. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3650, 3310, 1732, 1658, 1600; ^{1}H NMR: δ0.66 (3H, *s*, t-Me), 0.96 (3H, *s*, t-Me), 0.97 (3H, *d*, *J* = 5.5 Hz, 21-Me), 0.98 (3H, *s*, *t*-Me), 1.90 (3H, *s*, COMe), 2.25 [6H, *s*, $N(Me)_2$], 3.68 and 3.78 (1H, *d*, *J* = 10.0 Hz, 31-CH_2), 4.12 (1H, *m*, 6-H), 4.42 (1H, *m*, 3-H), 4.78 (1H, *m*, 16-H), 5.35 (1H, *dd*, 11-H), 5.66 (1H, *m*, 1-H), 5.96 (1H, *d*, NH), 7.33–7.78 (5H, 2*m*, ArH); MS *m/z* (rel. int.): 578 [M, 2]$^+$, 562 (1), 560 (1), 171 (2), 157 (2), 105 (15), 72 (100), 60 (1), 58 (3), 43 (3).

(−)-E-*Cyclobuxaphylamine* (**3**) *and* (−)-Z-*cyclobuxaphylamine* (**4**). Fraction A was loaded on a silica gel column (30 g), and eluted with C_6H_6–$CHCl_3$–Et_2NH (16:3:1). The important fractions were then purified by TLC (silica gel) to afford amorphous **3** (2.0 mg) and **4** (4.0 mg).

(−)-E-*Cyclobuxaphylamine* (**3**). $[\alpha]_D - 30$ ($CHCl_3$; *c* 1.89). UV λ_{max}^{MeOH} nm: 244 (log ε 3.81); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510, 1712, 1636, 1595; MS *m/z* (rel. int.): 367 [M, 30]$^+$, 352 (20), 338 (5), 138 (20), 84 (70), 71 (100), 58 (40).

(−)-Z-*Cyclobuxaphylamine* (**4**). $[\alpha]_D - 76$ ($CHCl_3$; *c* 1.11). UV λ_{max}^{MeOH} nm: 240 (log ε 3.70); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3515, 1709, 1637, 1600; MS *m/z* (rel. int.): 367 [M, 7]$^+$, 352 (22), 324 (8), 138 (30), 84 (12), 71 (100), 58 (80), 44 (25).

(+)-N-*Benzoylcycloxobuxine-F* (**5**). Fraction C was loaded on a silica gel column (70 g). Elution was with $CHCl_3$–MeOH–NH_4OH (90:9:1). Further purification was by TLC (silica gel) using the solvent system C_6H_6–C_6H_{14}–Et_2NH (10:10:1) to afford **5** (1.9 mg), amorphous, $[\alpha]_D + 50$ ($CHCl_3$; *c*

0.82); lit. $[\alpha]_D + 90°$ ($CHCl_3$; *c* 1.01) [16]; UV λ_{max}^{MeOH} nm: 226 (log ε 4.01); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 1655, 1650, 1595; MS *m/z* (rel. int.): 504 $[M, 1]^+$, 489 (2), 432 (2), 105 (15), 72 (100), 58 (20), 44 (3).

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