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STEROIDAL ALKALOIDS FROM *BUXUS PAPILLOSA*

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ABSTRACT.—New steroidal alkaloids, (+)-buxabenzacine [**1**] and (+)-buxafuranamide [**2**], have been isolated from the aerial parts of *Buxus papillosa*.

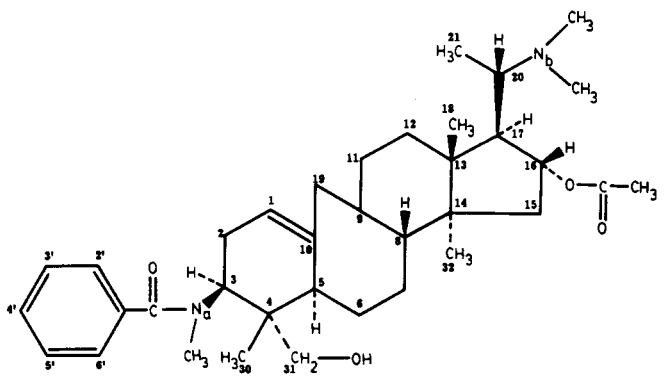
Buxus papillosa C.K. Schneider (Buxaceae) is a shrub widely distributed in the northern regions of Pakistan. Previous studies on *Buxus papillosa* have resulted in the isolation of a number of new steroidal alkaloids (1–4). Presently, we describe the isolation and structure elucidation of two new steroidal alkaloids, (+)-buxabenzacine [**1**] and (+)-buxafuranamide [**2**].

RESULTS AND DISCUSSION

(+)-Buxabenzacine [**1**], $C_{36}H_{54}N_2O_4$, showed uv absorption at 224 nm, characteristic of a tertiary benzamidic chromophore (5). The ir spectrum displayed intense bands at 3400 (OH), 1722 (ester carbonyl), 1626 (α,β-unsaturated carbonyl group), and 1617 (C=C) cm^{-1}.

The high resolution mass spectrum of (+)-buxabenzacine [**1**] displayed the molecular ion at *m/z* 578.3987, $C_{36}H_{54}N_2O_4$ (calcd 578.4083), indicating the presence of eleven double bond equivalents in the molecule. Peaks at *m/z* 171.1170 and 157.1099 were due to cleavage of ring D along with the nitrogen-containing side chain. These ions indicated that C-16 was the probable position of the acetate substituent on ring D. The peak at *m/z* 105 was due to the benzoyl ion. The substance showed a base peak at *m/z* 72 due to the trimethyliminium cation, $Me\text{-}CH{=}N^{+}(Me)_2$. The overall mass fragmentation pattern of compound **1** is very similar to the *Buxus* alkaloids bearing an *N*-dimethylamino group at C-20 and benzamide at C-3 (3).

The ^{1}H-nmr spectrum of (+)-buxabenzacine [**1**] featured three singlets for the three tertiary methyl groups at δ 0.80, 0.85, and 1.12, while a doublet centered at δ 0.82 was due to the C-21 secondary methyl group. The geminal C-20 proton appeared as a multiplet at δ 2.38. A three-proton singlet resonating at δ 2.00 was assigned to the acetyl methyl protons, while the six protons of the *N*-dimethyl group at C-20 appeared as a singlet at δ 2.12. A singlet at δ 2.75 was due to the methyl protons attached to the C-3 amidic nitrogen. A multiplet centered at δ 2.74 was assigned to the C-19



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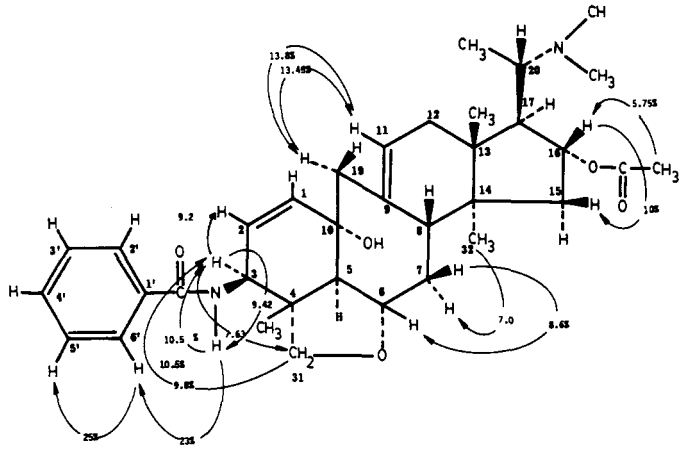

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methylenic protons. Two AB doublets resonating at δ 3.84 and 3.99 ($J_{31\alpha,31\beta}$ = 9.3 Hz) were due to C-31α and C-31β methylenic protons geminal to the hydroxyl group, respectively, while a multiplet at δ 4.73 was assigned to the C-16 methine proton geminal to the α-acetoxy group (5). A broad singlet at δ 5.62 assigned to the C-1 olefinic proton showed coupling with the C-2 methylene protons. This position was established by comparing the chemical shift of the C-1 olefinic proton with the value reported earlier (6). The five aromatic protons afforded multiplets centered at δ 7.38 (2H), 7.45 (1H), and 7.74 (2H).

The 2D-nmr measurements (2D *J*-resolved, COSY 45°, and NOESY) (7,8) also confirmed the above-mentioned assignments. The COSY-45° spectrum showed strong coupling interaction between the Me-21 (δ 0.82) and C-20 methine protons (δ 2.38). The interactions between the geminal C-31 methylenic protons (δ 3.84 and 3.99), and between the C-1 olefinic proton (δ 5.62) and the C-2 methylenic protons (δ 2.44) were also observed. The position of the double bond at C-1–C-10 was established by the mutual interactions of the olefinic C-1 proton (δ 5.62) as well as readily identifiable H-3 (δ 4.88) with the same intervening C-2 methylene protons (δ 2.44) in the COSY spectrum. Similarly, COSY interactions were also observed between H-19α and H-19β, between H-9 and H-19β, and between H-19α and H-16β/H-17α. The ^{13}C-nmr spectrum [broad-band and DEPT (9)] is summarized in Table 1. The chemical shift assignments are based on comparison with closely related compounds (14). Acetylation of **1** with Ac_2O/pyridine yielded the corresponding monoacetate, $C_{38}H_{56}N_2O_5$. The ^{1}H-nmr spectrum of the acetylated product showed a downfield shift of the 31-methylenic protons from δ 3.84 and 3.99 to δ 4.16 and 4.20, respectively. An additional 3H singlet appeared at δ 2.04 due to the 31-acetyl methyl protons. The rest of the ^{1}H-nmr spectrum closely resembled that of the parent alcohol, **1**. On the basis of the above studies, structure **1** was assigned to the new alkaloid, named (+)-buxabenzacine.

The second alkaloid, (+)-buxafuranamide [**2**], $C_{35}H_{48}N_2O_5$, showed uv absorption maxima at 228 nm characteristic of the secondary benzamidic chromophore (3,5). The ir spectrum displayed absorptions at 3650 (NH), 3410 (OH), 1716 (ester carbonyl), 1651 (amide carbonyl), 1595 (C=C), and 1210 (C-O stretch) cm^{-1}. The high resolution mass spectrum of **2** showed the molecular ion at *m/z* 576.3587 corresponding to the formula $C_{35}H_{48}N_2O_5$ and indicating the presence of thirteen double bond equivalents in the molecule. Another peak at *m/z* 516 resulted from the loss of HOAc. The peaks at *m/z* 171 and *m/z* 157 arose by the cleavage of ring D along with the attached substituents and therefore suggested C-16 as the probable position of the

TABLE 1. ^{13}C-nmr Assignments of (+)-Buxabenzacine [1] ($CDCl_3$).

Carbon	Multiplicity (DEPT)	Chemical shift[a] (δ)
C-1	CH	131.95
C-2	CH_2	35.58
C-3	CH	50.18
C-4	C	41.18
C-5	CH	53.47
C-6	CH_2	26.91
C-7	CH_2	30.35
C-8	CH	39.79
C-9	CH	38.72
C-10	C	134.14
C-11	CH_2	32.44
C-12	CH_2	35.13
C-13	C	44.51
C-14	C	49.05
C-15	CH_2	44.52
C-16	CH	78.02
C-17	CH	61.31
C-18	CH_3	17.15[b]
C-19	CH_2	35.13
C-20	CH	53.45
C-21	CH_3	9.63
C-30	CH_3	16.78[b]
C-31	CH_2	67.78
C-32	CH_3	17.43[b]
$N_b(CH_3)_2$	CH_3	39.78
N_a-C(=O)-	C	169.50
N_a-CH_3	CH_3	27.37
-O-C(=O)-CH_3	C	170.00
O-C(=O)-CH_3	CH_3	21.79
1′	C	134.48
2′	CH	127.07
3′	CH	128.52
4′	CH	131.95
5′	CH	128.52
6′	CH	127.07

[a]Multiplicities were confirmed by DEPT experiments and assignments are based on comparison with known compounds (13).
[b]Assignments are interchangeable.

acetoxy group on ring D. A large peak at *m/z* 105 was due to the benzoyl ion. A characteristic base peak at *m/z* 72 resulted from the cleavage of the ring D side chain. The overall mass fragmentation pattern was similar to closely related compounds reported earlier (3).

The ^{1}H-nmr spectrum ($CDCl_3$ + few drops of CD_3OD, 400 MHz) showed three tertiary methyl groups at δ 0.74, 0.92, and 1.15, while the secondary methyl group resonated as a doublet at δ 1.16. A 3H singlet at δ 1.95 was assigned to the acetate methyl group. Another 6H singlet at δ 2.57 was assigned to the protons of the two

TABLE 2. $^1H/^1H$ Connectivities from COSY-45 Spectrum of Buxabenzacine [1].

Proton	Chemical shift (δ)	Coupled to H (δ) (COSY-45°)
H-1	5.62	H-2 (2.44)
H-2	2.44	H-1 (5.62)
H-9	1.83	H-11β (1.54), H-19α (2.74), H-19β (2.70)
H-11β	1.54	H-9 (1.83)
H-11α	1.72	H-9 (1.83)
H-15β	1.53	H-16β (4.73)
H-15α	1.95	H-16β (4.73)
H-16β	4.73	H-15α (1.95), H-17α (2.05)
H-17α	2.05	H-16β (4.73)
H-19α	2.74	H-19β (2.70)
H-19β	2.70	H-19α (2.74)
H-20	2.38	H-21 (0.82)
Me-21	0.82	H-20 (2.38)
H-31α	3.84	H-31β (3.99)
H-31β	3.99	H-31α (3.84)

methyl groups attached to the nitrogen. A singlet resonating at δ 2.72 was due to the C-19 methylenic protons, its downfield chemical shift being consistent with its proximity to the olefinic bonds. A broad singlet at δ 5.47 was ascribed to the C-11 vinylic proton. The C-31α and C-31β methylene protons appeared as a set of doublets at δ 3.64 and 3.71 ($J_{31\alpha,31\beta} = 8.6$ Hz), respectively. A multiplet centered at δ 4.91 was assigned to the C-16 methine proton, adjacent to the acetoxy group. A doublet of doublets at δ 4.88 ($J_{3\alpha,NH} = 9.8$ Hz, $J_{3\alpha,2} = 3.1$ Hz) was due to the C-3 methine proton coupled to the amidic N-H and C-2 vinylic proton. The latter appeared as a doublet of doublets at δ 5.88 ($J_{2,1} = 10.3$ Hz). The neighboring C-1 vinylic proton appeared as a doublet at δ 5.86 ($J_{1,2} = 10.3$ Hz). The lack of any other coupling of the C-1 vinylic proton indicated the quaternary nature of C-10. A doublet at δ 6.63 ($J_{NH,3} = 9.8$ Hz) was due to the NH protons. The aromatic protons appeared as multiplets centered at 7.46 and 7.85. An interesting multiplet for the C-6 methine proton resonated at δ 4.51, its downfield shift reflecting the attachment of the ethereal oxygen to this carbon. It is relevant to point out at this stage that more than a dozen 6-hydroxycycloartenol triterpenoids (10) are known which possess an oxygen function at C-6 in ring B, as in (+)-buxafuranamide [**2**].

In order to determine the nature of the oxygen functions at C-6 and C-31, the ^1H-nmr spectrum was recorded in pyridine-d_5. It is known that under these conditions protons adjacent to hydroxyl or carbonyl groups suffer a pronounced paramagnetic shift (11). However, no significant changes in the chemical shifts of the C-6 or C-31 protons were observed, which further confirmed the ethereal nature of the oxygen atom attached to these carbons. The α stereochemistry of the C-4 oxygenated methylene is consistent with the results previously described on the basis of X-ray diffraction studies (12) on a related compound as well as the predicitions based on nmr studies (13). The above studies established structure **2** for (+)-buxafuranamide, which is the first member of a new group of steroidal bases with a pentacyclic skeleton having an extra ring formed between rings A and B by the cyclization of the C-31 hydroxymethylene with C-6. The nOe measurements were carried out to establish the relative stereochemistry at various centers. The results are summarized around expression **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were recorded on a Varian MAT 312 dou-

ble focussing mass spectrometer connected to a DEC PDP 11/34 computer system. The ^{1}H-nmr spectra were recorded in $CDCl_3$ with a few drops of CD_3OD on Bruker AM-400 nmr spectrometer at 400 MHz. The ^{13}C-nmr spectra were recorded at 100 MHz on the same instrument. The uv spectra were recorded on a Shimadzu UV-240 instrument. The ir spectra were recorded on a Jasco IRA-1 infrared spectrophotometer. Optical rotations were taken on a Polartronic D polarimeter. The melting point was recorded on Gallenkamp melting point apparatus and is uncorrected. The purity of the sample was checked on tlc (Si gel, precoated plates).

EXTRACTION AND FRACTIONATION.—The leaves of *B. papillosa* (40 kg) were collected in northern Pakistan. The plant was identified by plant taxonomist at the Department of Botany, University of Karachi, and a voucher specimen was deposited in the herbarium of the Department of Botany, University of Karachi. Extraction was with EtOH at room temperature. The solvent was evaporated in vacuo to afford a gum (110 g) that was taken up in 10% HOAc. The aqueous acidic extract was basified with NH_4OH and extracted with $CHCl_3$ at pH 5. The fraction obtained by extraction with $CHCl_3$ was loaded on an alumina column (Al_2O_3 GF 254, Type 60/E, E. Merck). The column was successively eluted with $CHCl_3$/MeOH mixtures in order of increasing polarity. Three main fractions were obtained: fraction A [$CHCl_3$-MeOH (95:5)] (15 g), fraction B [$CHCl_3$-MeOH (92:8)] (10 g), and fraction C [$CHCl_3$-MeOH (90:10)] (10 g).

ISOLATION OF (+)-BUXABENZACINE [**1**].—Fraction A (4 g) was placed on a Si gel column (200 g) (mesh 230–400) (5 × 30 cm). Elution was with hexane-$CHCl_3$-diethylamine (90:10:1). A fraction obtained was further purified using precoated plates (Si gel, SiF) in the same solvent system, which afforded a white crystalline alkaloid **1** (12 mg): $[\alpha]^{20}D$ +48°; uv λ max (MeOH) 224 nm; ir ν max ($CHCl_3$) 3400 (OH), 1722 (OAc), 1626 (C=C-CO), 1617 (-C=C-) cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$ + few drops of CD_3OD) δ 0.80 (3H, s, Me), 0.82 (3H, d, $J_{21,20}$ = 6.0 Hz, Me-21), 0.85 (3H, s, Me), 1.12 (3H, s, Me), 2.00 (3H, s, OAc), 2.12 (6H, s, N_b Me_2), 2.38 (1H, m, H-20), 2.44 (2H, m, H-2), 2.74 (2H, m, H_2-19), 2.75 (3H, s, N_a-Me), 3.84 (1H, d, $J_{31\alpha,31\beta}$ = 9.3 Hz, H-31α), 3.99 (1H, d, $J_{31\beta,31\alpha}$ = 9.3 Hz, H-31β), 4.73 (1H, m, H-16), 5.62 (1H, br s, H-1), 7.38–7.74 (5H, m, Ar-H); ms *m/z* (int %) [$C_{36}H_{54}N_2O_4$] 578.3987 (calcd 578.4083) (0.5%), [$C_{35}H_{51}N_2O_4$] 563.3476 (calcd 563.3484) (2%), [$C_9H_{17}NO_2$] 171.1170 (calcd 171.1259) (7%), [$C_8H_{15}NO_2$] 157.1099 (calcd 157.1102) (5%), [C_7H_5O] 105.0337 (calcd 105.0340) (42%), [$C_4H_{10}N$] 72.0812 (calcd 72.0813) (100%).

ACETYLATION OF (+)-BUXABENZACINE [**1**].—(+)-Buxabenzacine [**1**] (3 mg) was acetylated at room temperature using Ac_2O (0.1 ml) in pyridine (0.3 ml). After stirring for 24 h, the mixture was purified on tlc (Si gel) using C_6H_6-Me_2CO-$CHCl_3$-diethylamine (3:3:4.9:0.1) to afford the corresponding acetate: uv λ max (MeOH) 224 nm; ir ν max ($CHCl_3$) 1725 (ester carbonyl) cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) 0.85 (3H, s, Me), 0.88 (3H, s, Me), 0.93 (3H, d, $J_{21,20}$ = 7.0 Hz, Me-21), 1.18 (3H, s, Me), 1.93 (3H, s, OAc), 2.04 (3H, s, OCOMe), 2.05 (6H, s, NMe_2), 2.50 (3H, s, NMe), 4.16 (1H, d, $J_{31\alpha,31\beta}$ = 9.3 Hz, H-31α), 4.20 (1H, d, $J_{31\beta,31\alpha}$ = 9.3 Hz, H-31β), 4.78 (1H, m, H-16), 5.35 (1H, m, H-3α), 5.54 (1H, br s, H-1), 7.41–7.74 (5H, m, Ar-H); ms *m/z* (int. %) 620 (0.1), 605 (0.1), 171 (4), 105 (11), 73 (5), 72 (100), 58 (4), 57 (4).

ISOLATION OF (+)-BUXAFURANAMIDE [**2**].—Fraction B (2 g) was placed on a Si gel column (30 g) (mesh size 230–400). Elution was with hexane-$CHCl_3$-diethylamine (70:30:1). A fraction obtained therefrom was further purified (tlc, silica, SiF, precoated plates) in the same solvent system to supply **2** (10 mg). The compound was partially soluble in $CHCl_3$ and completely soluble in $CHCl_3$-MeOH (1:1). $[\alpha]^{24}D$ + 190°; uv λ max (MeOH) 228 nm; ir ν max ($CHCl_3$) 3650 (NH), 3410 (OH), 1716 (OAc), 1651 (amide carbonyl), 1595 (C=C), 1210 (C-O stretch) cm^{-1}; ^{1}H nmr ($CDCl_3$ + few drops of CD_3OD) δ 0.74 (3H, s, Me) 0.92 (3H, s, Me), 1.15 (3H, s, Me), 1.16 (3H, d, $J_{21,20}$ = 6.0 Hz, 21-Me), 1.52–1.85 (2H, m, H-7α/H-7β), 1.56–1.60 (2H, m, H-15α/H-15β), 1.96 (2H, m, H_2-12), 1.99 (1H, m, H-5α), 1.95 (3H, s, OAc), 2.05 (1H, m, H-17α), 2.57 (6H, br s, NMe_2), 2.62 (1H, m, H-20), 2.72 (2H, s, H-19), 3.64 (1H, d, $J_{31\alpha,31\beta}$ = 8.6 Hz, H-31α), 3.71 (1H, d, $J_{31\beta,31\alpha}$ = 8.6 Hz, H-31β), 4.51 (1H, m, H-6), 4.88 (1H, dd, $J_{3\alpha NH}$ = 9.8 Hz, $J_{3\alpha,2}$ = 3.1 Hz, H-3α), 4.91 (1H, m, H-16), 5.47 (1H, br s, H-11), 5.86 (1H, d, $J_{1,2}$ = 10.3 Hz, H-1), 5.88 (1H, dd, $J_{2,1}$ = 10.3 Hz, $J_{2,3\alpha}$ = 3.1 Hz, H-2); 6.63 (1H, d, $J_{NH,3\alpha}$ = 9.8 Hz, NH) 7.46–7.85 (5H, m, Ar-H); ms *m/z* (rel. int.): [M]$^+$ 576.3587 ($C_{35}H_{48}N_2O_5$) (calcd 576.4079) (75%), 561 (85%), 516 (25%), 171 (12%), 157 (5%), 105 (40%), 85 (36%), 72 (100%).

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