New Steroidal Alkaloids from the Roots of Buxus papillosa

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NEW STEROIDAL ALKALOIDS FROM THE ROOTS OF *BUXUS PAPILLOSA*

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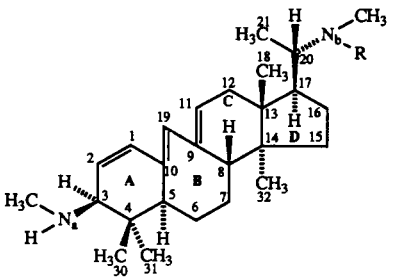
ABSTRACT.—The roots of *Buxus papillosa* have yielded three new steroidal alkaloids, (+)-papillotrienine [**1**], (+)-N_b-demethylpapillotrienine [**2**], and (+)-N_b-demethylharappamine [**3**]. The structures have been determined on the basis of spectroscopic studies.

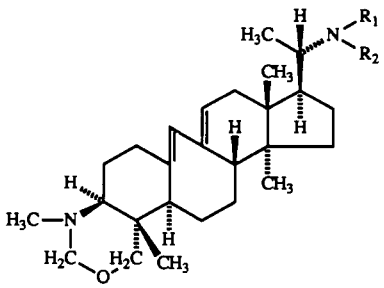
Buxus papillosa C.K. Schneider (Buxaceae) is a shrub widely distributed in the northern regions of Pakistan and locally known as Shamshad (1). Extracts of the shrub find extensive use in the indigenous system of medicine for the treatment of various disorders, especially malaria, rheumatism, and skin infections. We have previously reported a number of new steroidal alkaloids from the leaves of *B. papillosa* (2–7). We report here the isolation and structure elucidation of three new steroidal alkaloids, papillotrienine [**1**], N_b-demethylpapillotrienine [**2**], and N_b-demethylharappamine [**3**]. The structures have been determined through extensive spectroscopic studies. Compounds **1** and **2** are members of a rare group of *Buxus* bases having conjugated triene chromophores in rings A, B, and C.

RESULTS AND DISCUSSION

(+)-Papillotrienine [**1**], $C_{27}H_{44}N_2$, showed a uv absorption maximum at 289 nm, characteristic of a triene system (8). The ir spectrum displayed absorptions at 3300 (N-H) and 1600 (C=C) cm^{-1}. The ^{1}H-nmr spectrum ($CDCl_3$, 400 MHz) showed four three-proton singlets at δ 0.71, 0.89, 0.80, and 0.93, corresponding to the four tertiary Me groups. A doublet centered at δ 1.32 ($J_{21,20}$ = 7.3 Hz) was due to the C-21 secondary Me protons. Another three-proton singlet at δ 2.13 was assigned to the *N*Me protons, while the $N(Me)_2$ protons appeared as a singlet at δ 2.80. A one-proton multiplet at δ 3.42 was ascribed to the C-3 methine proton. The olefinic H-1 appeared as a doublet at δ 5.58 ($J_{1,2}$ = 9.9 Hz), while the olefinic H-11 resonated as a broad singlet at δ 5.71 (8). A one-proton singlet at δ 6.11 was assigned to the olefinic H-19, whereas a doublet of doublet centered at δ 6.25 ($J_{2,1}$ = 10.2 Hz, $J_{2,3}$ = 1.9 Hz) was due to the olefinic H-2 (8).



1 R=Me
2 R=H



3 R_1=R_2=H
4 R_1=H, R_2=Me

The COSY 45° spectrum of **1** showed cross peaks of H-1 (δ 5.58) with H-2 (δ 6.25). The olefinic H-2 showed interaction with the methine H-3 (δ 3.42), whereas COSY 45° interaction between H-3α and H-5α (δ 2.56) was also observed. These observations further confirmed the above mentioned assignments.

The ^{13}C-nmr spectrum ($CDCl_3$) of **1** showed four signals at δ 11.37, 15.10, 16.56, and 16.91 due to C-21, C-30, C-18, and C-31. C-1 resonated at δ 128.65, while C-2 appeared at δ 129.24. C-11 and C-19 resonated at δ 126.82 and 127.60 respectively. The complete ^{13}C-nmr chemical shift assignments are presented in Table 1.

The hreims of **1** showed the molecular ion at *m/z* 396.3513, corresponding to the molecular formula, $C_{27}H_{44}N_2$ (calcd 396.3504), indicating the presence of seven degrees of unsaturation in the molecule. The peak at *m/z* 381.3265 resulted from the loss of an Me group from the molecular ion. The peak at *m/z* 283.2295 ($C_{20}H_{29}N$, calcd 282.2299) arose due to the cleavage of ring D. The base peak at *m/z* 72.0812 ($C_4H_{10}N$, calcd 72.0813) arose by the cleavage of the nitrogen-containing side chain on ring D (9). On the basis of these data structure **1** was assigned to this new alkaloid.

The second compound, (+)-N_b-demethylpapillotrienine [**2**], $C_{26}H_{42}N_2$, was also isolated from roots of *B. papillosa*. The ^{1}H-nmr spectrum of **2** ($CDCl_3$, 400 MHz) was found to be virtually identical to that of **1**, except for the absence of the N_b-Me group. The peak at δ 2.21 integrating for three protons was assigned to the C-20 *N*-Me protons.

TABLE 1. ^{13}C-Nmr ($CDCl_3$) Chemical Shift Assignments of **1** and **3**.

Carbon	Compound	
	1	**3**
C-1	128.65 (CH)	39.34 (CH_2)
C-2	129.24 (CH)	26.02 (CH_2)
C-3	68.41 (CH)	71.23 (CH)
C-4	40.11 (-C-)	38.50 (-C)
C-5	46.68 (CH)	48.52 (CH)
C-6	24.89 (CH_2)	25.36 (CH_2)
C-7	41.00 (CH_2)	25.57 (CH_2)
C-8	46.67 (CH)	49.47 (CH)
C-9	136.75 (-C-)	136.04 (-C-)
C-10	138.75 (-C-)	134.50 (-C-)
C-11	126.82 (CH)	129.70 (CH)
C-12	30.68 (CH_2)	38.37 (CH_2)
C-13	44.06 (-C-)	43.20 (-C)
C-14	49.36 (-C-)	49.70 (-C-)
C-15	30.68 (CH_2)	33.34 (CH_2)
C-16	25.49 (CH_2)	29.02 (CH_2)
C-17	51.06 (CH)	49.75 (CH)
C-18	16.56 (CH_3)	17.37(CH_3)
C-19	127.60 (CH)	130.11 (CH)
C-20	56.38 (CH)	58.94 (CH)
C-21	11.37 (Me)	14.00 (Me)
C-30	15.10 (Me)	18.89 (Me)
C-31	16.91 (Me)	78.08 (CH_2)
C-32	17.60 (Me)	19.31 (Me)
C-33	—	88.48 (CH_2)
N_a Me	38.36 (Me)	36.35 (Me)
N_b Me	40.11 (Me)	—

The ms of **2** showed the molecular ion at *m/z* 382.3346 ($C_{26}H_{42}N_2$, calcd 382.3348), 14 mu less than compound **1**. The overall mass fragmentation pattern corresponded to that of compounds **1** and **2**, with many of the fragments appearing 14 mu below corresponding peaks in **2** as expected for the N_b-demethyl analogue. The base peak at *m/z* 58, having the formula C_3H_8N, arose due to the cleavage of the D ring nitrogen-containing side chain (9). In light of these data, structure **2** was assigned to this new alkaloid.

Compounds **1** and **2** belong to a unique group of *Buxus* alkaloids having a conjugated triene system. These compounds may arise in nature by elimination of a suitable leaving group at C-1 or C-2 of the precursor *abeo*-diene alkaloidal system. Compounds **1** and **2** have also been isolated by us recently from roots of *Buxus sempervirens* of Turkish origin.

The third compound, (+)-N_b-demethylharappamine [**3**], $C_{26}H_{42}N_2O$ (found 398.3313, calcd 398.3297), showed uv absorption maxima at 238 and 246 nm, characteristic of a 9(10↦19)*abeo*-diene system (10,11). Its ir spectrum displayed absorptions at 3600 (N-H) and 1590 (C=C) cm^{-1}. The ^{1}H-nmr spectrum ($CDCl_3$, 400 MHz) displayed three-proton singlets at δ 0.70, 0.74, and 1.03, corresponding to the three tertiary Me groups. A doublet centered at δ 1.20 ($J_{21,20}$ = 8.0 Hz) was assigned to the C-21 secondary Me protons. A three-proton singlet at δ 2.12 was assigned to the NH Me group of the tetrahydrooxazine ring. A set of AB doublets resonating at δ 3.25 and δ 3.82 ($J_{31\alpha,31\beta}$ = 10.6 Hz) were assigned to the C-31 methylenic protons while another set of AB doublets at δ 3.59 and 4.44 (J = 7.6 Hz) were due to methylenic protons of the NH-CH_2-O- moiety. A broad singlet at δ 5.54 was assigned to the vinylic H-11, while a singlet at δ 5.97 was due to the isolated vinylic H-19. Complete assignments of the ^{13}C-nmr spectrum are shown in Table 1.

The hreims of compound **3** showed the molecular ion at *m/z* 398.3313 (calcd 398.3297), corresponding to the molecular formula $C_{26}H_{42}N_2O$. A large peak at *m/z* 354.2793, having the formula $C_{24}H_{36}NO$ (calcd 354.2796), arose due to the cleavage of the C-17 nitrogen-containing side chain. Another peak at *m/z* 341.2716 corresponding to the formula $C_{23}H_{35}NO$ (calcd 341.2718) was due to the cleavage of ring D. The base peak at *m/z* 127.9961 having the formula $C_7H_{13}NO$ (calcd 127.9970) was due to the cleavage of ring A along with the *N*-methyl tetrahydrooxazine ring-containing side chain. These studies led to the assignment of structure **3** to this new base.

Compound **3** belongs to a class of *Buxus* alkaloids that contain a tetrahydrooxazine or Me-substituted tetrahydrooxazine ring in their structures, and its overall spectral behavior was distinctly similar to an earlier reported compounds, harappamine [**4**] and papillamidine (12,13). The formation of the tetrahydrooxazine ring may proceed in nature by the condensation of formaldehyde or acetaldehyde with the C-3 amino group. The attack of the C-4β hydroxymethylene on the corresponding ketimine can result in the formation of the tetrahydrooxazine ring.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H-nmr spectra were recorded at 400 MHz in $CDCl_3$, and ^{13}C-nmr spectra were recorded at 75.4 MHz in $CDCl_3$. Ms were recorded on a Varian MAT 112S mass spectrometer connected to a DEC PDP 11/34 computer system. Hreims were recorded on a Jeol-JMS HX 110 mass spectrometer. Tlc experiments were performed on Si gel (GF-254) precoated plates (E. Merck).

PLANT MATERIAL.—The roots of *B. papillosa* (100 kg) were collected from the northern areas of Pakistan in December 1989. The plant was identified by Prof. Syed Irtifaq Ali, Department of Botany, University of Karachi. A voucher specimen was deposited in the herbarium of the Department of Botany, University of Karachi, Karachi 75270, Pakistan.

EXTRACTION AND ISOLATION.—The EtOH extract of the air-dried roots (100 kg) was evaporated

to a gum (500 g). The alkaloids (100 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 9 (40 g) was loaded on a Si gel column (300 g), and elution was carried out first with $CHCl_3$ (500 ml) and then with $CHCl_3$/MeOH (500 ml). Several fractions were obtained, and a fraction selected for further studies was designated as fraction A [$CHCl_3$-MeOH, (10:2)]. The fraction obtained at pH 3 (30 g) was loaded on to a Si gel column (300 g), and a fraction obtained on elution with $CHCl_3$-MeOH (10:1) was designated as fraction B.

(+)-*Papillotrienine* [**1**].—Fraction A was subjected to repeated preparative tlc using petroleum ether-Me_2CO-Et_2NH (25:5:0.5) on Si gel plates of 0.2 mm thickness to afford (+)-papillotrienine [**1**] as a white amorphous solid (15 mg): $[\alpha]^{20}D$ 60° ($CHCl_3$); uv λ max (MeOH) nm 289; ir ν max ($CHCl_3$) cm^{-1} 3300 (N-H), 1600 (C=C); ^1H nmr ($CDCl_3$, 400 MHz) δ 0.71 (3H, s, Me), 0.80 (3H, s, Me), 0.89 (3H, s, Me), 0.93 (3H, s, Me), 1.32 (3H, d, $J_{21,20}$ = 7.3 Hz, Me-21), 2.13 (3H, s, NMe), 2.80 (6H, s, N Me_2), 3.42 (1H, m, H-3), 5.58 (1H, d, $J_{1,2}$ = 9.9 Hz, H-1), 5.71 (1H, m, H-11), 6.11 (1H, s, H-19), 6.25 (1H, dd, $J_{2,3}$ = 1.9 Hz, $J_{2,1}$ = 10.2 Hz; H-2); hreims *m/z* (rel. int. %) $[M]^+$ 396.3513 ($C_{27}H_{44}N_2$ calcd 396.3504) (100), $[M-Me]^+$ 381.3265 ($C_{26}H_{41}N_2$ calcd 381.3269) (2), 283.2295 ($C_{20}H_{29}N$ calcd 283.2299) (9), 72.0812 ($C_4H_{10}N$ calcd 72.0813) (100).

(+)-N_b-*Demethylpapillotrienine* [**2**].—Fraction A was subjected to repeated preparative tlc using petroleum ether-Me_2CO-Et_2OH (25:5:0.5) to afford N_b-demethylpapillotrienine [**2**] as a white amorphous solid (20 mg): $[\alpha]^{20}D$ 62° ($CHCl_3$); uv λ max (MeOH) nm 289; ir ν max ($CHCl_3$) cm^{-1} 3350 (NH), 1595 (C=C) cm^{-1}; ^1H-nmr ($CDCl_3$, 400 MHz) δ 0.77 (3H, s, Me), 0.79 (3H, s, Me), 0.84 (3H, s, Me), 1.17 (3H, s, Me), 1.33 (3H, d, $J_{21,20}$ = 6.5 Hz, Me-21), 2.21 (6H, s, N_a Me, N_b Me), 3.56 (1H, m, H-3), 5.58 (1H, d, $J_{1,2}$ = 10 Hz, H-1), 5.84 (1H, m, H-11), 6.23 (1H, s, H-19), 6.33 (1H, dd, $J_{2,3}$ = 2.2 Hz, $J_{2,1}$ = 10.0 Hz, H-2); hreims *m/z* (rel. int. %) $[M]^+$ 382.3346 ($C_{26}H_{42}N_2$ calcd 382.3348) (48), $[M-Me]^+$ 367.3198 ($C_{25}H_{39}N_2$ calcd 367.3113) (10), 311.2612 ($C_{22}H_{33}N$ calcd 311.2613) (10), 58.0694 (C_3H_8N calcd 58.0657) (100).

(+)-N_b-*Demethylharappamine* [**3**].—Fraction B (30 g) was subjected to chromatography on a Merck Lobar column (Lobar Si 60), and elution was carried out first with $CHCl_3$ (20 ml), then with $CHCl_3$-MeOH-Et_2NH (10:0.5:0.05) (20 ml) to afford (+)-N_b-demethylharappamine [**3**] as colorless amorphous solid (30 mg): $[\alpha]^{20}D$ 12.4° ($CHCl_3$); uv λ max (MeOH) nm 238, 246; ir ν max ($CHCl_3$) cm^{-1} 3600 (NH), 1590 (C=C); ^1H nmr ($CDCl_3$, 400 MHz) δ 0.70 (3H, s, Me), 0.74 (3H, s, Me), 1.03 (3H, s, Me), 1.20 (3H, d, $J_{21,20}$ = 8.0 Hz, Me-21), 2.12 (3H, s, NMe), 3.25 (1H, d, J = 10.6 Hz, H-31α), 3.59 (1H, d, J = 7.6 Hz, H-33α), 3.82 (1H, d, J = 10.6 Hz, H-31β), 4.44 (1H, d, J = 7.6 Hz, H-33β), 5.54 (1H, s, H-11), 5.97 (1H, s, H-19); hreims *m/z* (rel. int. %) $[M]^+$ 398.3313 ($C_{26}H_{42}N_2O$ calcd 398.3297) (84), $[M-Me]^+$ 383.3066 (calcd 383.3062) (64), 354.2793 ($C_{24}H_{36}NO$ calcd 354.2796) (24), 341.2716 ($C_{23}H_{35}NO$ calcd 341.2718) (20), 127.9961 ($C_7H_{13}NO$ calcd 127.9970) (100).

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