

NEW TRITERPENOIDAL ALKALOIDS FROM THE LEAVES OF *BUXUS PAPILLOSA*

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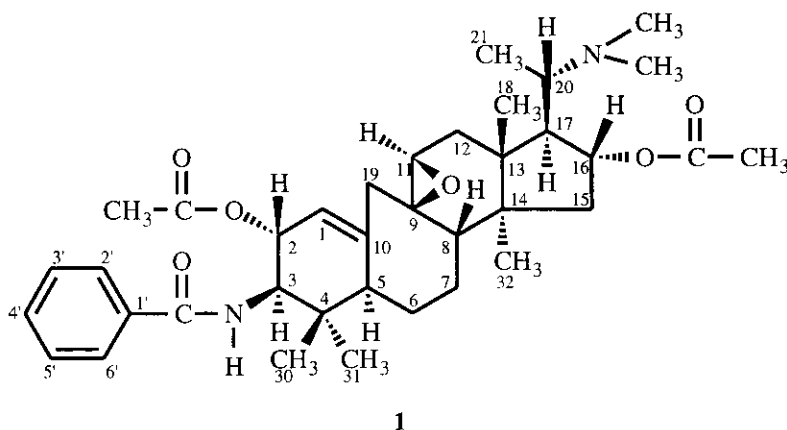
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Abstract- Phytochemical investigations on the ethanolic extract of the leaves of *Buxus papillosa* of Pakistani origin have resulted in the isolation of two new triterpenoidal alkaloids, (-)-2 α ,16 α -diacetoxy-9 β ,11 β -epoxybuxamidine (**1**) and (+)-papillozine-C (**2**) along with one known triterpenoidal base, (-)-sempervirone (**3**) isolated for the first time from this species. The structures of these natural products were established through detailed spectroscopic studies.

Buxus papillosa C.K. Schneider (Buxaceae) is widely distributed in North West Province of Pakistan (NWFP). The crude extract of the plant finds extensive use in the treatment of various ailments such as rheumatism, skin disorders, and venereal diseases in the indigenous system of medicines.¹ Our previous phytochemical studies on this species have resulted in the isolation of over thirty new triterpenoidal alkaloids.²⁻⁴ Our recent studies on the chemical constituents on the leaves of *B. papillosa* have yielded two new triterpenoidal alkaloids, (-)-2 α ,16 α -diacetoxy-9 β ,11 β -epoxybuxamidine (**1**) and (+)-papillozine-C (**2**) along with one known triterpenoidal base, (-)-sempervirone (**3**) isolated for the first time from this species. The structures of these compounds were elucidated with the aid of extensive spectroscopic studies.

(-)-2 α ,16 α -Diacetoxy-9 β ,11 β -epoxybuxamidine (**1**) was isolated as a colorless amorphous solid. The UV spectrum showed maximum absorption at 228 nm indicating the presence of a secondary benzamide chromophore.⁵ The IR spectrum displayed intense absorption bands at 3314 (NH), 1706 (ester carbonyl), 1654 (α , β -unsaturated amide carbonyl), 1596 (C=C) and 1145 (C-O-C) cm⁻¹.

The high-resolution electron-impact mass spectrum (HREIMS) of **1** showed the molecular ion peak at m/z 620.3830 which provided molecular formula C₃₇H₅₂N₂O₆ (calcd 620.3825) and indicated the presence of thirteen degrees of unsaturation in the molecule. Ion at m/z 605.3597 (C₃₆H₄₉N₂O₆, calcd 605.3590) was due to the loss of a methyl group from the molecular ion. Another ion at m/z 105.0342 (C₇H₅O, calcd 105.0340) was representative of the benzoyl group. The base peak at m/z 72.0811 (C₄H₁₀N, calcd 72.0813) indicated a dimethyl-imminium cation formed by the cleavage of C-17/C-20 bond with charge retention on the nitrogen side chain.⁶ Other important ions at m/z 171.1246 (C₉H₁₇NO₂, calcd 171.1259), 157.1101 (C₈H₁₅NO₂, calcd 157.1103), and 85.0886 (C₅H₁₁N, calcd 85.0891) arose due to the cleavage of ring D along with the nitrogen containing side chain and suggested the presence of an acetoxy group at C-16.⁷



The $^1\text{H-NMR}$ spectrum (CDCl_3 , 500 MHz) of **1** showed resonances for the four three-proton singlets at δ 0.74, 0.78, 0.85 and 1.05 due to the four tertiary methyl groups. A three-proton doublet at δ 0.98 ($J_{21,20} = 6.7$ Hz) was ascribed to the secondary methyl protons. Two singlets, integrating for three protons each resonating at δ 1.82 and 1.93 were attributed to the methyl protons of the two acetyl groups substituted at C-2 and C-16, respectively. The *N,N*-dimethyl protons appeared as a six-proton singlet at δ 2.27 while the allylic C-19 methylene protons resonated as two-proton broad singlet at δ 2.78. A one-proton doublet at δ 3.02 ($J_{11\alpha,12\beta} = 10.6$ Hz, $J_{11\alpha,12\alpha} = 3.9$ Hz) was due to the C-11 methine proton, geminal to the epoxide moiety. The C-3 methine proton geminal to the benzamide functionality resonated at δ 4.05 ($J_{3\alpha,2\beta} = 9.8$ Hz, $J_{3\alpha,\text{NH}} = 9.6$ Hz). A downfield one-proton multiplet at δ 4.68 was due to the C-16 methine proton, while the C-2 methine proton appeared as a broad doublet at δ 5.37 ($J_{2\beta,3\alpha} = 9.8$ Hz). The downfield chemical shift of C-2 and C-16 reflected the presence of a geminal acetoxy group. The C-1 olefinic proton appeared at δ 5.40. The exchangeable amidic NH resonated at δ 6.10 ($J_{\text{NH},3\alpha} = 9.6$ Hz). This signal disappeared when $^1\text{H-NMR}$ spectrum was recorded in CD_3OD .⁸ The signals at δ 7.30 ($J_1 = 7.8$ Hz, $J_2 = 7.4$ Hz), 7.45 ($J_1 = 7.8$ Hz, $J_2 = 1.3$ Hz) and 7.63 ($J_1 = 7.4$ Hz, $J_2 = 1.3$ Hz) were ascribed to *meta* C-3'/5', *para* C-4' and *ortho* C-2'/6' aromatic protons of the benzamide functionality, respectively.

The COSY-45 $^\circ$ and HOHAHA spectra⁹⁻¹⁰ of **1** revealed the presence of five spin systems "a~e" in the molecule (Figure 1). The spin system "a" consists of a phenyl moiety. The spin system "b" starts with the C-1 olefinic proton (δ 5.40) which exhibited vicinal couplings with the C-2 methine proton (δ 5.37). The latter in turn showed cross-peaks with the C-3 methine proton (δ 4.05) and COSY-45 $^\circ$ interactions with the amidic NH (δ 6.10). Allylic couplings of the C-1 olefinic proton with the C-19 methylene (δ 2.78) and C-5 methine (δ 2.50) protons were also observed in the HOHAHA spectra. The spin system "c" begins with the C-5 methine proton (δ 2.50) which showed COSY-45 $^\circ$ interactions with the C-6 methylene protons (δ 1.72 and 1.45). The latter showed vicinal couplings with the C-7 methylene protons (δ 1.54 and 1.29). The C-7 methylene protons also showed cross-peaks with the C-8 methine proton (δ 1.80). The fourth spin system "d" includes the C-11 methine proton (δ 3.02) which showed $^1\text{H-}^1\text{H}$ shift correlations with the C-12 methylene protons (δ 1.68 and 1.34). The fifth spin system "e" was traced out from the spin couplings of the C-16 methine proton (δ 4.68) with the C-15 methylene (δ 1.77 and 1.49) and the C-17 methine (δ

2.49) protons. The latter showed vicinal coupling with the C-20 methine proton (δ 2.39) which in turn exhibited cross-peaks with the C-21 methyl protons (δ 0.98). The HOHAHA spectra (20 ms, 60 ms and 100 ms) showed long-range homonuclear couplings between all the coupled protons of each spin system.

The ^{13}C -NMR spectrum (CDCl_3 , 125 MHz) of **1** showed the resonances of all thirtyseven carbon atoms in the molecule. DEPT spectra⁹ revealed the presence of fourteen methine, five methylene and nine methyl carbons. The subtraction of DEPT spectra from the broad-band spectrum indicated the presence of nine quaternary carbon atoms, in the molecule. The ^{13}C -NMR spectrum contained two aliphatic downfield signals at δ 77.1 and 70.2 due to the C-2 and C-16 carbon atoms, respectively. Their downfield value reflected the presence of a geminal acetoxy group. The olefinic C-1 resonated at δ 131.5. The complete ^{13}C -NMR chemical shift assignments to each carbon atoms of **1** are presented in Table 1. The inverse one-bond shift correlation NMR experiment (HMQC) established the direct $^1\text{H}/^{13}\text{C}$ one-bond connectivity of each protonated carbon atom in the molecule.⁹ The HMQC spectrum showed $^1\text{H}/^{13}\text{C}$ one-bond shift correlations of H-2 (δ 5.37) and H-16 (δ 4.68) with C-2 (δ 77.1) and C-16 (δ 70.2) respectively. The olefinic H-1 (δ 5.40) exhibited HMQC interactions with the C-1 (δ 131.5). Similarly $^1\text{H}/^{13}\text{C}$ one-bond shift correlations of each protonated of the carbon atoms in compound **1** as determined from HMQC spectrum are shown in Table 1.

The inverse long-range $^1\text{H}/^{13}\text{C}$ shift correlation experiment (HMBC)⁹ was very useful for the ^{13}C -NMR chemical shift assignments of quaternary carbon atoms and in the establishment of structure **1** from various sub-structures "a-e" obtained from the COSY-45 $^\circ$ and HOHAHA spectra. H-2'/6' (δ 7.63) (spin system "a") showed long-range interactions with the C-1' (δ 132.9) and the amidic carbonyl (δ 169.1). The C-3 methine proton (δ 4.05) (spin system "b") also exhibited HMBC interactions with the amidic carbonyl (δ 169.1). These observations suggested that C-1' (spin system "a") was linked with C-3 (spin system "b") through an amide bond. The C-1 olefinic (δ 5.40) and C-3 methine (δ 4.05) (spin system "b") showed cross-peaks with the C-10 (δ 128.3) and C-4 (δ 53.2) quaternary carbon atoms, respectively. H-5 (δ 2.50) (spin system "c") also exhibited long-range heteronuclear interactions with C-10 (δ 128.3) and C-4 (δ 53.2). The C-30 methyl (δ 0.78) and C-31 methyl (δ 0.85) protons also showed cross-peaks with the C-4 quaternary carbon atom. These observations indicated that C-1 and C-3 (spin system "b") were linked with C-5 (spin system "c") through quaternary C-10 and C-4 atoms, respectively. H-11 (δ 3.02) (spin system "d") showed HMBC interactions with the quaternary C-9 (δ 62.1). The C-19 methylene (δ 2.78) and C-8 methine (δ 1.80) (spin system "c") protons showed cross-peaks with C-9. These HMBC interactions helped to connect C-11 (spin system "d") with C-19 and C-8 (spin system "c") through the C-9 quaternary carbon atom. The C-12 methylene (δ 1.68 and 1.34) (spin system "d") and C-17 methine (δ 2.49) protons (spin system "e") exhibited cross-peaks with C-13 (δ 44.4). The C-8 methine (δ 1.80) (spin system "c") and C-15 methylene (δ 1.77 and 1.49) protons (spin system "e") showed heteronuclear multiple bond connectivity with C-14 (δ 38.7). These HMBC observations linked C-12 (spin system "d") with C-17 (spin system "e"), and C-8 (spin system "c") with C-15 (spin system "e") through the C-13 and C-14 quaternary carbon atoms respectively to establish structure **1**.

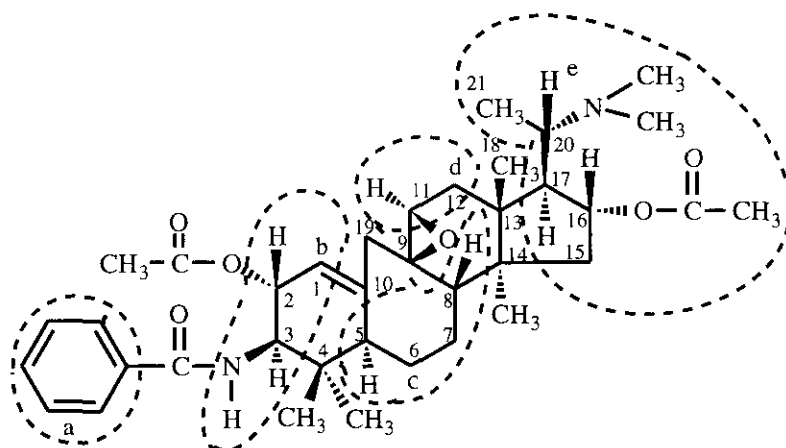


Figure 1: Spin systems "a"~"e" obtained from COSY-45° and HOHAHA spectra of **1**.

The stereochemistry at various chiral centers was established on the basis of ^1H - ^1H -coupling constants and the NOESY spectrum. The α -stereochemistry of C-2/ OAc was established on the basis of the *trans* diaxial coupling between the C-2 H β and C-3 H α ($J_{2\beta,3\alpha} = 9.8$ Hz). The β -stereochemistry for the C-9/C-11 epoxide was deduced on the basis of the NOESY spectrum in which H-11 α (δ 3.02) showed cross-peaks with H-5 (δ 2.50) which in turn exhibited NOE interactions with H-3 (δ 4.05). H-3 and H-5 are invariably α -oriented while H-8 is always β -oriented in this class of alkaloids.¹⁰ The probable conformations of various rings and important NOE interactions are shown in Figure 2. Based on these spectroscopic studies, structure **1** was established for this new triterpenoidal alkaloid.

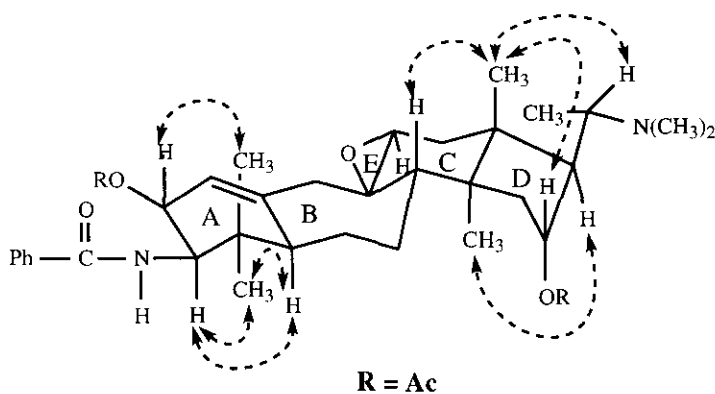


Figure 2: Probable conformation and important NOE interactions of **1** as obtained from NOESY spectrum.

(+)-Papillozine-C (**2**) was isolated as a colorless amorphous solid. The UV spectrum contained absorption maxima at 237 and 244 nm with shoulders at 219 and 240 nm, characteristic of a 9(10 \rightarrow 19) *abeo* diene system commonly found in *Buxus* alkaloids.¹¹ The IR spectrum afforded intense absorptions at 3395 (NH), 2860 (CH), 1690 (conjugated C=C) and 1100 (C-O-C) cm^{-1} .

Table 1 ^{13}C -NMR Chemical Shift Assignments of **1** and **2** and $^1\text{H}/^{13}\text{C}$ One-Bond Shift Correlations of each Protonated Carbon Atoms of **1** as Determined from HMQC Spectrum

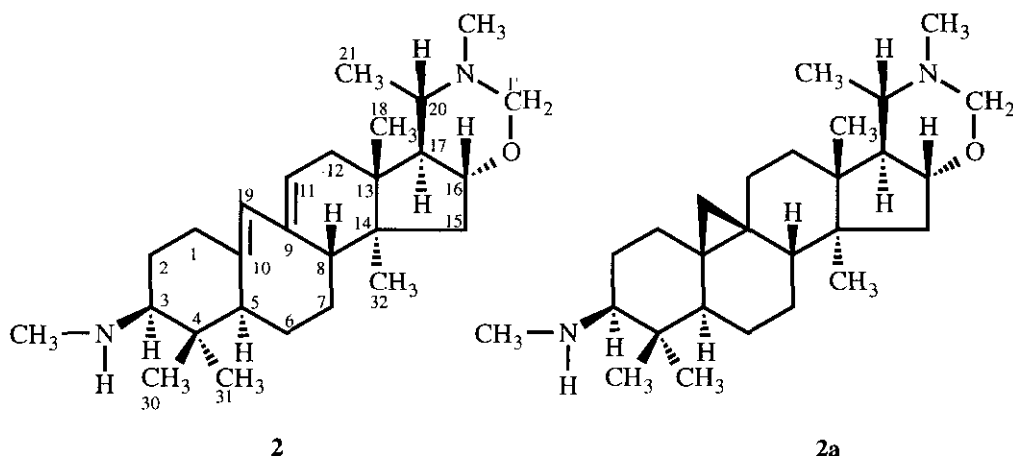
Carbon No.	1			2	
	^{13}C δ	Multiplicity [†]	^1H δ	^{13}C δ	Multiplicity [†]
C-1	131.5	CH	5.40	34.4	CH ₂
C-2	77.1	CH	5.37	30.1	CH ₂
C-3	59.1	CH	4.05	69.2	CH
C-4	53.2	-C-	---	40.9	-C-
C-5	53.5	CH	2.50	49.5	CH
C-6	26.6	CH ₂	1.72,1.45	25.4	CH ₂
C-7	35.4	CH ₂	1.54,1.29	27.5	CH ₂
C-8	41.2	CH	1.80	49.8	CH
C-9	62.1	-C-	---	133.4	-C-
C-10	128.3	-C-	---	134.1	-C-
C-11	65.8	CH	3.02	130.1	CH
C-12	35.2	CH ₂	1.68,1.34	38.5	CH ₂
C-13	44.4	-C-	---	44.5	-C-
C-14	38.7	-C-	---	45.7	-C-
C-15	37.4	CH ₂	1.77,1.49	33.0	CH ₂
C-16	70.2	CH	4.68	77.3	CH
C-17	50.4	CH	2.49	51.4	CH
C-18	16.7	CH ₃	0.74	14.3#	CH ₃
C-19	44.5	CH ₂	2.78	132.2	CH
C-20	61.1	CH	2.39	62.0	CH
C-21	11.1	CH ₃	0.98	9.8	CH ₃
C-30	15.2	CH ₃	0.78	15.4#	CH ₃
C-31	17.1	CH ₃	0.85	16.9#	CH ₃
C-32	17.4	CH ₃	1.05	17.3#	CH ₃
N _a -CH ₃	---	---	---	42.1	CH ₃
N _b -CH ₃	39.6	CH ₃	2.27	38.9	CH ₃
OCOCH ₃	21.4	CH ₃	1.82	---	---
OCOCH ₃	21.9	CH ₃	1.93	---	---
OCOCH ₃	170.4	-C-	---	---	---
OCOCH ₃	171.8	-C-	---	---	---
NHCO	169.1	-C-	---	---	---
C-1'	132.9	-C-	---	85.2	CH ₂
C-2'	127.5	CH	7.63	---	---
C-3'	129.0	CH	7.30	---	---
C-4'	134.0	CH	7.45	---	---
C-5'	129.0	CH	7.30	---	---
C-6'	127.5	CH	7.63	---	---

[†]Determined by DEPT spectra

#Assignments are interchangeable

The high-resolution electron-impact MS spectrum of **2** showed the molecular ion peak at m/z 412.3456 (calcd 412.3453) appropriate for the molecular formula $\text{C}_{27}\text{H}_{44}\text{N}_2\text{O}$ indicating the presence of seven degrees of unsaturation in the molecule. The ion at m/z 397.3217 ($\text{C}_{26}\text{H}_{41}\text{N}_2\text{O}$, calcd 397.3218) was due

to the loss of a methyl group from the molecular ion. The MS fragmentation pattern of **2** was substantially different from that of the dibasic *Buxus* alkaloids with the molecular ion appearing as a base peak, thus suggesting the modification of the α -amino C-17 side chain, while the fragmentation at C-3 remains unchanged. It has already been reported in the literature that *Buxus* alkaloids containing an *N*-methyl or an *N,N*-dimethyl functionality at C-20 exhibit the base peak at m/z 58 (C_3H_8N) or 72 ($C_4H_{10}N$) respectively. Compound **2** however showed M^+ as base peak while other ions at m/z 127.0997 ($C_7H_{13}NO$) and m/z 113 ($C_6H_{11}NO$) arose probably by the cleavage of ring D and suggested the presence of a tetrahydrooxazine moiety at C-16.¹² Ions at m/z 57 (C_3H_7N) and 71 (C_4H_9N) can arise by the cleavage of ring A and indicate the presence of an *N*-methyl group at C-3.⁶



The 1H -NMR spectrum ($CDCl_3$, 400 MHz) of **2** afforded four three-proton singlets at δ 0.61, 0.66, 1.01 and 1.17 due to the four tertiary methyl groups. Another three-proton doublet at δ 1.19 ($J_{21,20} = 6.5$ Hz) was due to the C-21 secondary methyl protons. Two broad singlets integrating for three protons each appeared at δ 2.23 and 2.37 and were ascribed to the N_a - and N_b - methyl protons respectively. A set of two AB doublets, integrating for one proton each appeared at δ 3.87 and 4.10 ($J_{1'a,1'b} = 10.8$ Hz) due to the methylene protons flanked by two heteroatoms i.e. nitrogen and oxygen atoms of the tetrahydrooxazine ring while the C-16 methine appeared as one-proton multiplet at δ 4.45. Two olefinic signals integrating for one proton each at δ 5.52 (broad singlet) and 5.92 (sharp singlet), due to the C-11 and C-19 olefinic protons respectively, were also observed in the 1H -NMR spectrum.

The 1H -NMR chemical shift assignments were further supported by two dimensional NMR studies using shift correlation spectroscopy (COSY-45 $^\circ$). The C-3 methine proton (δ 2.63) showed spin-spin coupling with the C-2 methylene protons (δ 2.05 and 1.60) which in turn exhibited cross-peaks with the C-1 methylene protons (δ 2.15 and 1.35). The vinylic C-11 proton (δ 5.52) showed vicinal couplings with the C-12 methylene protons (δ 2.15 and 1.90) in the COSY-45 $^\circ$ spectrum. The C-16 methine proton (δ 4.45) exhibited COSY-45 $^\circ$ interactions with the C-15 methylene (δ 2.06 and 1.79) and C-17 methine (δ 3.73) protons. The latter showed vicinal coupling with the C-20 methine proton (δ 1.25) which in turn exhibited 1H - 1H -shift correlations with the C-21 methyl protons (δ 1.19). The C-1' methylene protons also exhibited

geminal coupling between them but no vicinal coupling was observed for these methylene protons which further suggested that C-1' methylene protons are flanked by two heteroatoms.

The ^{13}C -NMR spectrum (CDCl_3 , 100 MHz) of **2** showed resonances for all twentyseven carbon atoms. DEPT spectra revealed the presence of eight methine, seven methylene and seven methyl carbon atoms while the subtraction of DEPT spectra from the broad band spectrum indicated the presence of five quaternary carbon atoms in the molecule. The ^{13}C -NMR spectrum showed a downfield aliphatic signal at δ 85.2 due to the C-1' methylene carbon sandwiched between the nitrogen and oxygen atoms of the tetrahydrooxazine moiety. Another signal at δ 77.3 was due to C-16, geminal to the oxygen functionality. The olefinic carbon resonances at δ 132.2 and 130.1 were ascribed to C-19 and C-11, respectively. Complete ^{13}C -NMR chemical shift assignments to all carbon atoms of compound **1** are presented in Table 1.

A NOESY spectrum was also recorded to deduce the stereochemistry at various chiral centers. The probable conformations of rings A,B,C,D and E as deduced from NOESY spectrum and important NOE interactions are shown in Figure 3. These spectroscopic studies led to structure (**2**) for this new triterpenoidal base.

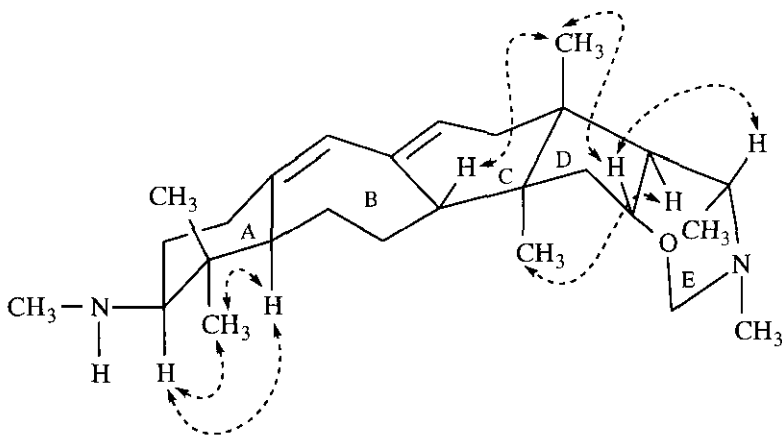
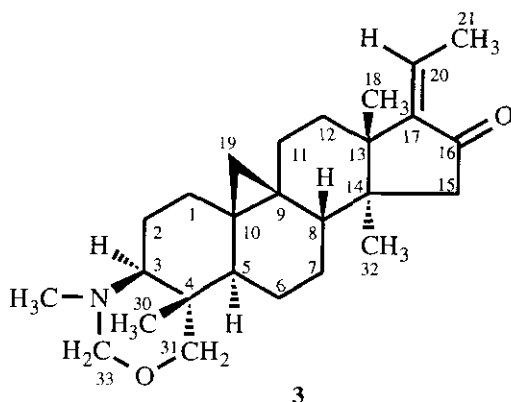


Figure 3: Probable conformation and important NOE interactions of **2** as deduced from the NOESY spectrum.

Compound (**2**) is the second example of *Buxus* alkaloids containing a tetrahydrooxazine ring incorporated in their structures at C-16/C-20 and its overall spectral behavior was distinctly similar to an earlier reported alkaloid, (+)-buxozine-C (**2a**).¹² The latter contains a C-19 cyclopropyl system while compound **2** bears a 9(10→19) *abeo* diene system. The formation of the tetrahydrooxazine ring may occur in nature by the condensation of formaldehyde with the C-20 amino group. The attack of the C-16/ α OH on the corresponding ketimine can result in the formation of the tetrahydrooxazine ring. A number of biogenetic precursors *i.e.* *Buxus* alkaloids having C-16/ α OH and C-20/HN-CH₃ functionalities have already been

reported by our research group.²

In addition to these new natural products, one known triterpenoidal alkaloid (-)-sempervirone (**3**) isolated for the first time from this species of genus *Buxus* was also isolated as a colorless amorphous solid. The UV, IR, ¹H-NMR and MS spectra of **3** were similar to those of (-)-sempervirone reported in the literature.¹³ Compound (**3**) was previously isolated from *Buxus sempervirens*.¹³



3

EXPERIMENTAL

General: The MS spectra were recorded on a Varian MAT 112 mass spectrometer connected to a DEC PDP 11/34 computer system. HREIMS were recorded on a Jeol-JMS HX 110 mass spectrometer. ¹H-NMR spectra were recorded in CDCl₃ or CD₃OD on Bruker AM 400 and Bruker AM 500 instruments at 400 MHz and 500 MHz, while ¹³C-NMR spectra were also recorded in CDCl₃ on the same instruments at 100 MHz and 125 MHz. UV and IR spectra were recorded on Shimadzu UV 240 and Shimadzu IR 240 spectrophotometers, respectively. The optical rotations were measured on a JASCO DIP-360 digital polarimeter. The purity of the samples was checked by TLC (Merck silica gel precoated plates having 0.5 mm thickness).

Plant Material: The leaves of *Buxus papillosa* (100 kg) were collected from North West Frontier Province (NWFP) area of Pakistan during March 1991. The plant was identified by Dr. Tahir Ali, plant taxonomist and a specimen voucher (# KUH 66486) has been deposited in Herbarium, Department of Botany, University of Karachi.

Extraction and Isolation: The leaves of *Buxus papillosa* (100 kg) were dried and extracted with methanol (100 L) for five days at room temperature. The methanolic extract was evaporated under reduced pressure to afford a gum (2.25 kg). This gum was dissolved in distilled water (1.0 L). This aqueous extract was extracted with chloroform at different pH values *i.e.* 3.5, 7.0 and 9.5. The chloroform extract (600 g) obtained at pH 9.5 was subjected to vacuum liquid chromatography (VLC) over silica gel using pet. ether (40-60°), pet. ether (40-60°)-chloroform (1:1), chloroform, chloroform-methanol (1:1) and methanol as

solvent systems to afford five major fractions. The VLC fraction (76 g) obtained on elution with pet. ether(40-60°)-chloroform (1:1) was loaded onto a silica gel column (500 g). Elution was carried out with pet. ether (40-60°)-chloroform (0-100%) and chloroform-methanol (0-100%) to afford various fractions. A fraction obtained on elution of silica gel column with 10% methanol-90% chloroform was subjected to preparative TLC using pet. ether (40-60°)-ether-diethylamine (9:1:0.1) as the solvent system to yield (-)-2 α ,16 α -diacetoxy-9 β ,11 β -epoxybuxamidine (**1**) (R_f = 0.65) as a colorless amorphous solid (45 mg, 4.5×10^{-3} % yield).

Another VLC fraction (132 g) which was obtained on elution with chloroform-methanol (1:1) was also loaded onto a silica gel column (500 g) which was again eluted with pet. ether (40-60°) with increasing amounts of chloroform, chloroform with increasing amounts of methanol, and methanol. Two fractions F-1 and F-2 were obtained on elution of the silica gel column with 20% methanol-80% chloroform and 30% methanol-70% chloroform, respectively. The preparative TLC of fractions F-1 and F-2 using pet-ether(40-60°)-ether-diethylamine (8.5:1.5:0.1) and pet. ether (40-60°)-acetone-diethylamine (6:4:0.1) afforded (+)-papillozine-C (**2**) (R_f = 0.35, 26 mg, 2.6×10^{-6} % yield) and (-)-sempervirone (**3**), R_f = 0.44, (21 mg, 2.1×10^{-6} % yield) as colorless amorphous solids, respectively.

(-)-2 α ,16 α -Diacetoxy-9 β ,11 β -epoxybuxamidine (1): Colorless amorphous solid (45 mg, 4.5×10^{-3} % yield); $[\alpha]_D^{20} = -76^\circ$ ($c = 1.2$, CHCl_3); UV λ_{max} (MeOH) 228 nm; IR (CHCl_3) ν_{max} cm^{-1} : 3314 (NH), 1706 (ester carbonyl), 1654 (α , β -unsaturated amide carbonyl), 1596 (C=C), 1145 (C-O-C); $^1\text{H-NMR}$ (CDCl_3) δ : See Table 1; $^{13}\text{C-NMR}$ (CDCl_3) δ : See Table 1; HREIMS m/z (rel. int. %) 620.3830 ($\text{C}_{37}\text{H}_{52}\text{N}_2\text{O}_6$, calcd 620.3825, 0.6), 605.3597 ($\text{C}_{36}\text{H}_{49}\text{N}_2\text{O}_6$, calcd 605.3590, 12.4), 171.1246 ($\text{C}_9\text{H}_{17}\text{NO}_2$, calcd 171.1259, 19.6), 157.1101 ($\text{C}_8\text{H}_{15}\text{NO}_2$, calcd 157.1103, 29), 105.0342 ($\text{C}_7\text{H}_5\text{O}$, calcd 105.0340, 45), 85.0886 ($\text{C}_5\text{H}_{11}\text{N}$, calcd 85.0891, 11), 72.0811 ($\text{C}_4\text{H}_{10}\text{N}$, calcd 72.0813, 100).

(+)-Papillozine-C (2): Colorless amorphous solid (26 mg, 2.6×10^{-6} % yield); $[\alpha]_D^{20} = +23^\circ$ ($c = 0.5$, CHCl_3); UV λ_{max} (MeOH) 219 (s), 237, 240 (sh), 244 nm; IR (CHCl_3) ν_{max} cm^{-1} : 3395 (NH), 2860 (CH), 1690 (conjugated C=C) and 1100 (C-O-C); $^1\text{H-NMR}$ (CDCl_3) δ : 0.61 (3H, s, CH_3), 0.66 (3H, s, CH_3), 1.01 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.19 (3H, d, $J_{21,20} = 6.5$ Hz, 21- CH_3), 2.23 (3H, br s, N_a - CH_3), 2.37 (3H, br s, N_b - CH_3), 3.87 (1H, d, $J_{1'a,1'b} = 10.8$ Hz, H-1'a), 4.10 (1H, d, $J_{1'b,1'a} = 10.8$ Hz, H-1'b), 4.45 (1H, m, H-16 β), 5.52 (1H, br s, H-11), 5.92 (1H, s, H-19); $^{13}\text{C-NMR}$ (CDCl_3) δ : See Table 1; HREIMS m/z (rel. int. %) 412.3456 ($\text{C}_{27}\text{H}_{44}\text{N}_2\text{O}$, calcd 412.3453, 100), 397.3217 ($\text{C}_{26}\text{H}_{41}\text{N}_2\text{O}$, calcd 397.3218, 85), 127.0997 ($\text{C}_7\text{H}_{13}\text{NO}$, calcd 127.0997, 34), 113.0844 ($\text{C}_6\text{H}_{11}\text{NO}$, calcd 113.0840, 29), 71.0733 ($\text{C}_4\text{H}_9\text{N}$, calcd 71.0735, 45), 57.0574 ($\text{C}_3\text{H}_7\text{N}$, calcd 57.0578, 73).

(-)-Sempervirone (3): Colorless amorphous solid (21 mg, 2.1×10^{-6} % yield); $[\alpha]_D^{20} = -123^\circ$ ($c = 0.61$, CHCl_3); UV λ_{max} (MeOH) 243 nm; IR (CHCl_3) ν_{max} cm^{-1} : 2916 (CH), 1712 and 1634 (α , β -unsaturated cyclopentanone); $^1\text{H-NMR}$ (CDCl_3) δ : 0.38 (1H, d, $J_{19\alpha,19\beta} = 4.5$ Hz, H-19 α), 0.65 (1H, d, $J_{19\beta,19\alpha} = 4.5$ Hz, H-19 β), 0.94 (3H, s, CH_3), 1.16 (3H, s, CH_3), 1.29 (3H, s, CH_3), 1.84 (3H, d, $J_{21,20} = 7.5$ Hz, 21- CH_3), 2.19 (3H, s, N_a - CH_3), 3.16 (1H, d, $J_{31\alpha,31\beta} = 10.6$ Hz, H-31 α), 3.63 (1H,

d, $J_{33\alpha,33\beta} = 7.5$ Hz, H-33 α), 3.77 (1H, d, $J_{31\beta,31\alpha} = 10.6$ Hz, H-31 β), 4.46 (1H, d, $J_{33\beta,33\alpha} = 7.5$ Hz, H-33 β), 6.55 (1H, q, $J_{20,21} = 7.5$ Hz, H-20); HREIMS m/z (rel. int. %) 397.2980 (C₂₆H₃₉NO₂, calcd 397.2981, 15), 382.2741 (C₂₅H₃₆NO₂, calcd 382.2741, 24), 127.0997 (C₇H₁₃NO, calcd 127.0997, 100).

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