BUXAPAPILININE - A NOVEL ALKALOID FROM THE LEAVES OF BUXUS PAPILLOSA

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Abstract–A novel alkaloid buxapapilinine (1) has been isolated from the leaves of Buxus papillosa. Its structure has been investigated by extensive nmr studies.

Buxus papillosa C.K. Schneider (Buxaceae) is a shrub growing extensively in the northern regions of Pakistan. Buxus extracts have been used in the indigenous system of medicine for the treatment of malaria, rheumatism and skin diseases.[1] Previous studies on Buxus papillosa have resulted in the isolation of a number of steroidal alkaloids.[2-5] As a consequence of our continuing studies on the leaves of Buxus papillosa, a novel base buxapapilinine has been isolated, to which structure **1** has been assigned on the basis of extensive nmr studies.

The crude alkaloids were isolated from the concentrated alcoholic extract of the leaves of Buxus papillosa by extraction at different pH values. The $CHCl_3$ extract obtained by extraction at pH 3.8 was concentrated and subjected to column chromatography. Further purification by preparative tlc resulted in the isolation of compound **1** as a white crystalline solid.

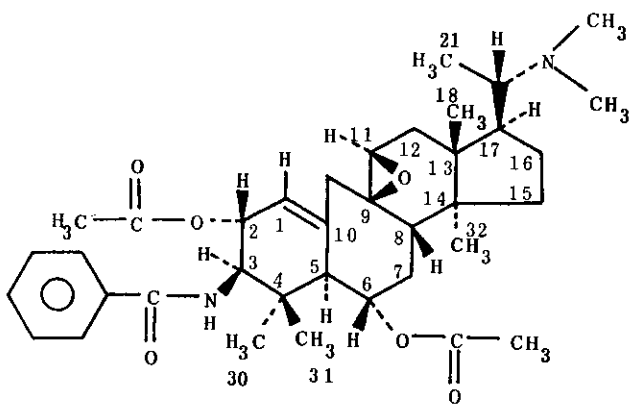

Figure 1

The compound showed uv absorption at 228 nm characteristic of the benzamidic chromophore.[5-6] The ir spectrum showed an intense absorptions at 2950 (NH), 1730 (ester carbonyl), 1670 (amide carbonyl) and 1240 (C-O-C) cm^{-1}. The mass spectrum of compound **1** showed the molecular ion

peak at m/z 620.3862 corresponding to the molecular formula $C_{37}H_{52}N_2O_6$ (calcd. 620.3833). A peak at m/z 605.3601 ($C_{36}H_{49}N_2O_6$) appeared due to the loss of methyl group from the molecular ion. Another important fragment at m/z 175.1011 with the composition $C_{11}H_{13}NO$ was due to the retro Diels-Alder cleavage of ring A along with the attached substituents. Similarly the peak at m/z 105.0810 (C_7H_5O) was attributed to the benzoyl ion. The compound showed a base peak at m/z 72.0824 corresponding to the composition $C_4H_{10}N$, representing trimethyl iminium side chain cleavage. The overall mass fragmentation pattern of compound 1 was found to be similar to related Buxus alkaloids bearing N,N-dimethyl group at C-20 and benzamide at C-3 position.[4]

The ^{1}H-nmr spectrum (400 MHz, $CDCl_3$), homodecoupling experiments, NOE difference, COSY-45°, and hetero-COSY experiments, all contributed to the deduction of the structure of the new alkaloid. Four three-proton singlets at δ 0.84, 0.88, 0.93 and 1.17 were assigned to the four tertiary methyl groups. A doublet at δ 0.82 ($J_{21,20}$ = 6.0 Hz) was ascribed to C-21 secondary methyl protons. The three-proton singlets resonating at δ 1.93 and δ 2.04 were assigned to the acetyl methyl protons of the acetate groups at C-6 and C-2 respectively, while the six protons of N-dimethyl group at C-20 appeared as a broadened singlet at δ2.16. A broad multiplet at δ 2.41 was assigned to the C-20 methine proton. Another multiplet centered at δ 4.79 was due to C-6 proton which is geminal to the acetate function.[6] The C-2βH showed a double doublet at δ 5.54 ($J_{2\beta,3\alpha}$ = 8.8 Hz, $J_{2\beta,1}$ = 0.6 Hz) while C-3αH resonated at δ 4.12. The equatorial (C-3β) disposition of the amide functionality is consistent with biogenetic considerations, since no steroidal alkaloids with axially oriented nitrogen function at C-3 have been previously reported. The axial disposition (α-orientations) of C-3H is consistent with the trans diaxial coupling of 8.8Hz observed between C-3αH and C-2βH, thereby establishing the equatorial (α-) orientation of the C-2 acetate groups. The sterically hindered amide NH appeared as a doublet at δ 6.20 ($J_{NH,3\alpha}$ = 9.8 Hz). A broad singlet at δ5.54 was due to C-1 vinylic protons. A doublet at δ 3.01 was assigned to the C-11 proton, its downfield chemical shift reflecting the presence of an epoxide at C-9/C-11. Assignments for the other protons are given in Table I.

In the COSY-45° spectrum coupling[7] of C-11αH (δ3.01) was observed with C-19αH and C-19βH (δ 2.79 and 1.80 assignments interchangeable), as well as C-12αH and C-12βH (δ 1.77 and δ 1.49 assigments interchangeable). The signal for C-3αH appeared at δ 4.12 and was coupled with C-2βH (δ 5.54) as well as with the amide proton at δ6.20. The assignment of C-6βH at δ 4.79 was confirmed by its COSY interaction with C-5 αH (δ 2.65), the latter also showing allylic coupling with the C-1 olefinic proton (δ 5.54). The C-20 methine proton showed coupling with C-17αH (δ 1.80). The upfield value of C-17βH confirmed that no substituent was present at C-16. The COSY-45° spectrum is presented in Table I. A series of homodecoupling experiments were also carried out to further verify the interrelationships between the coupled protons.

The ^{13}C-nmr spectrum (broadband and DEPT, 100 MHz, $CDCl_3$)[8] showed the presence of 37 carbon atoms in the molecule. Multiplicity assignments by DEPT established that there were nine CH_3, five CH_2, fourteen CH, and nine quaternary carbon atoms. The signal for the vinylic C-1 appeared at δ129.44 while the signal for C-2 methine carbon appeared at δ 70.01, its downfield chemical shift reflecting the presence of an acetate group. Similarly another signal at δ77.80 was assigned to C-6 due to the presence of another acetate function. The C-11 methine and C-9 quaternary carbon showed signals at δ 63.47 and 62.09 respectively corresponding to the presence of an epoxide functionality. Similarly another downfield methine carbon signal observed at δ61.14 was assigned to the C-20 bearing the dimethylamino group. The ^{13}C-nmr chemical shift assignments were made with the help of hetero-COSY and DEPT measurements are presented in Table II.

In the one-bond hetero-COSY spectrum the methine carbon signals at δ 129.44 (C-1),δ77.80 (C-6), δ70.03 (C-2),δ63.47 (C-11),δ61.14 (C-20),δ59.33 (C-3) andδ53.51 (C-5) showed connectivities with their respective protons at δ 5.54 (C-1H),δ4.79 (C-6βH),δ5.54 (C-2βH), δ3.01 (C-11 H)δ2.41 (C-20 βH), δ 4.12 (C-3α H) and δ2.65 (C-5 αH) respectively while the methylene proton signals at δ 2.79 (C-19βH), δ 1.80 (C-19αH) showed conectivities with the carbon at δ 44.52. The ^{1}H - ^{13}C ($^{1}J_{CH}$) assignments to other centres are presented in Table II.

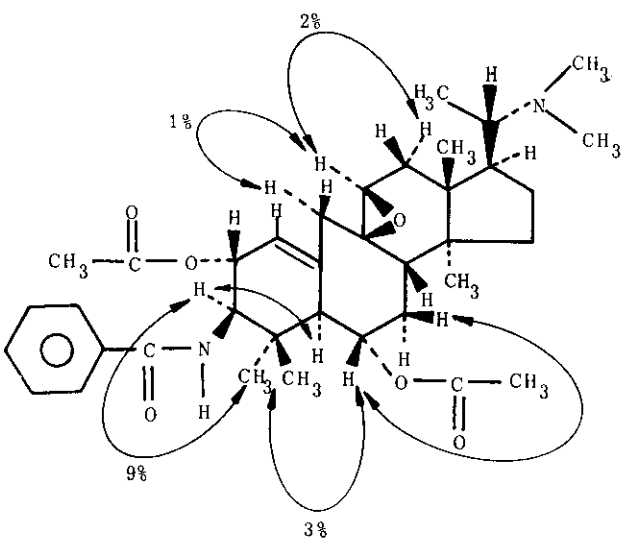

Figure 2: NOE interaction in Buxapaplimine

Table-1: $^{1}H/^{1}H$ Connectivities from COSY-45 spectrum of buxapapilinine

^{1}H	Chemical shift (δ)	Coupled to H (δ) (COSY-45)	^{1}H	Chemical shift(δ)	coupled to H (δ) (COSY45)
C-1H	5.54	-	C-16βH*	1.54	16αH (1.83) 13 H (1.40)
C-2βH	5.54	3αH(4.12)	C-17αH	1.80	16αH (1.85)
C-3αH	4.12	2βH(5.52), NH(6.2)	C-18CH_3**	0.84	
C-5αH	2.65	6βH(4.79)	C-19βH*	1.80	19αH (2.79)
C-6βH	4.79	5αH (2.65)	C-19αH*	2.79	19βH (1.80), 11 H (3.01)
C-7αH	2.03	7βH (1.63)	C-20βH	2.41	21CH_3 (0.82)
C-7αH	1.63	7βH (2.03)	O$COCH_3$	1.93	-
C-8βH	2.15		O$COCH_3$	2.04	-
C-11αH	3.01	12βH (1.99), 19 H (1.80)	NH	6.20	3αH (4.12)
C-12β$_H$*	1.99	12αH (1.77) 11 H (3.01)	C-30 CH_3**	1.17	-
C-12βH*	1.77	12βH (1.99)	C-31 CH_3**	0.93	-
C-15αH*	1.25	15αH (1.40)	C-32CH_3**	0.88	
C-15βH*	1.40	15βH (1.25), 16 H (1.54)	C-21CH_3(Sec)	0.82	
C-16αH*	1.83	16βH(1.54), 17 H (1.80)			

*,** Assignments interchangeable.

Table-II: ^{13}C-Nmr and ^{1}H - ^{13}C connectivities (Hetero-COSY)

C.No.	Chemical shift	Multiplicity (DEPT)	Connected to H(δ)	C.No.	Chemical shift	Multiplicity (DEPT)	Connected to H(δ)
1	129.44	CH	(5.54)	17	50.41	CH	αH(1.80)
2	70.03	CH	βH(5.52)	18	17.52	CH_3	(0.84)
3	59.33	CH	αH (4.12)	19	44.52	CH_2	βH(1.80),αH(2.79)
4	39.01	-C-		20	61.14	CH	H (2.41)
5	53.51	CH	βH(2.65)	21	9.61	CH_3	(0.82)
6	77.86	CH	βH(4.79)	O$\underline{C}OCH_3$	21.04	CH_3	(1.92)
7	35.57	CH_2	βH(2.03) H(1.63)	O$CO\underline{C}H_3$	21.85	CH_3	(2.04)
8	41.16	CH	αH(2.15)	O$\underline{C}OCH_3$	169.73	-C-	
9	62.09	-C-		O$\underline{C}OCH_3$	171.85	-C-	
10	134.42	-C-		=C-N	167.89	-C-	
11	63.47	CH	αH (3.01)	2-o,ArC	128.69	CH	(7.42)
12	35.21	CH_2	βH(1.77) H(1.99)	2-m,ArC	126.82	CH	(7.73)
13	42.83	-C-		p,ArC	131.57	CH	(7.49)
14	49.16	-C-		CH_3	39.94	CH_3	(2.16)
15	27.01	CH_2	βH(1.40), H(1.25)	Ar1O	134.2	-C-	
16	32.50	CH_2	βH(1.83) H (1.54)	30	27.31	CH_3	(1.18)
				32	17.52	CH_3	(0.84)

NOE difference studies served to establish the stereochemistry at various asymmetric centres. Irradiation at δ 4.79 (C-6βH) caused 3% and 2.5% nOe effects at the C-31 methyl protons and C-7β H respectively. On the other hand irradiation at δ 1.77 (C-12αH) showed 2% nOe at δ 3.01, assigned to C-11α H. Similarly irradiation at δ 2.79 (C-19βH) also showed 2% nOe on C-11αH which therefore confirmed the β stereochemistry of the epoxide. Other important nOe interactions are presented around Figure 2.

Hydrolysis of compound 1 with 6% KOH/MeOH yielded the corresponding diol, $C_{33}H_{48}N_2O_4$. The ^{1}H-nmr spectrum ($CDCl_3$, 300 MHz) of the hydrolyzed product showed relatively upfield shift of the C-6 and C-2 protons from δ 4.79 and 5.52 to δ 3.72 and 4.12 respectively. Consequently the neighbouring protons at C-5 and C-3 have also been shifted upfield (i.e. from δ 2.65 and δ 4.12 to δ2.15 and δ3.90 respectively). The rest of the proton spectrum closely resembles that of the parent acetate 1. The above assignments were further confirmed with COSY-45° experiments. It was observed that the signal for C-3αH at δ 3.90 was coupled with C-2βH (δ 4.12) as well as with the amide proton at δ6.20. The C-6βH which resonated at δ 3.72 was coupled with C-5αH (δ 2.15), and it also showed homoallylic coupling with the C-1 olefinic proton (δ5.54). The rest of the interactions is similar to those of the parent compound.

EXPERIMENTAL

Mass spectra were recorded on Varian MAT 312 double-focussing spectrometer connected to a DEC PDP 11/34 computer system. ^{1}H-Nmr spectra were recorded in $CDCl_3$ on Bruker AM-400 instrument at 400 MHz while ^{13}C-nmr spectra were recorded on the same instrument at 100 MHz. Ultraviolet spectra were recorded on a Shimadzu uv 240 spectrophotometer. Infrared spectra was recorded on JASCO ir spectrophotometer. The purity of the sample was checked on tlc (silica gel precoated plates). The plant material was identified by the plant taxonomist in the Department of Botany, University of Karachi, where a voucher specimen of the plant has been deposited.

The fresh leaves of Buxus papillosa (50 kg) collected from the nothern area of Pakistan were extracted 3 times with EtOH (100 l) at room temperature for 3 weeks respectively. The combined ethanol extract was evaporated to a gum (1 kg) and partitioned to yield n-hexane, chloroform and aqueous extracts. The bases in the chloroform solution were extracted with 10% AcOH partial separation of the alkaloids (10 g) was carried out by extraction in $CHCl_3$ at different pH values. The fraction obtained at pH 3.8 (25 g) was loaded on a silica gel column (70-230 mesh, AsTM Merck). Elution with $CHCl_3$-MeOH (9.0:1.0) afforded a number of close moving alkaloids. The mixture was subjected to prep. tlc (silica gel) employing hexane: $CHCl_3$:Et_2NH (7: 3 : 0.5) to afford an amorphous compound **1** (10.3 mg), $[\alpha]_D^{20}$ = - 28° (c 0.1, $CHCl_3$), uv λ_{max} (MeOH) 228 nm; ir (KBr) ν_{max} cm^{-1} 2950 (N-H), 1730 (O-$\overset{O}{\overset{\|}{C}}$-$CH_3$), 1670 (C-$\overset{O}{\overset{\|}{N}}$H), 1240 (C-O-C); ^{1}H-nmr (400 MHz, $CDCl_3$) : (Table II); ^{13}C-nmr (100 MHz, $CDCl_3$) : (Table-II); Hrms m/z (%): 620.3862

($M,^+$ calcd for $C_{37}H_{52}N_2O_6$: 620.3833) (18), 605.36016 ($M^+ -CH_3$ calcd for $C_{36}H_{49}N_2O_6$, 605.9534) (16), 489.28757 (M^+ calcd for $C_{27}H_{41}N_2O_6$ 489.2873) (40), 105.0810 (M^+ calcd for C_8H_9O, 105.0711) (50) 175.10115 (M^+ calcd for $C_{11}H_{13}NO$, 175.09970) (36), 72.0824 (M^+ calcd for $C_4H_{10}N$, 72.0814) (100).

Hydrolysis of Buxapapilinine

Buxapapilinine (5 mg) was hydrolysed at room temperature using 6% ethanolic KOH (5 ml) and refluxed for 24 h. The normal workup of the reaction product involving acidification, extraction with chloroform, and drying with anhyd. Na_2SO_4 afforded an amorphous alcohol **2**(1.2 mg) on evaporation: uv λ_{max} (MeOH) 228 nm; ir ν_{max} ($CHCl_3$) 3400 (OH) cm^{-1}; ^{1}H-nmr (300 MHz, $CDCl_3$) : δ 0.84 (3H, s, CH_3-18), 1.17 (3H,s, CH_3-30), 0.93 (3H, s, CH_3-31), 0.88 (3H, s, CH_3-32), 0.82 (3H, d, $J_{21,20}$ = 6.0 Hz, CH_3-21), 2.16 (6H, s, $N(CH_3)_2$), 1.80 (1H, d, $J_{17\alpha,20}$ = 10.6 Hz, Hα-17), 2.14 (1H, dd, $J_{20\beta,21}$ = 6.2 Hz, $J_{20\beta,17\beta}$= 10.3Hz Hβ-20), 3.72 (1H, m, Hβ-6), 4.12 (1H, m, Hβ-2), 5.54 (1H, s, H-1), 3.90 (1H, dd, $J_{3\alpha,2\beta}$= 8.0 Hz, $J_{3\alpha,NH}$ = 9.2 Hz, Hα-3), 6.20 (1H, d, $J_{NH,3\alpha}$= 9.8 Hz, NH); ms m/z 536 (12%), 521 (15%), 175 (36%), 157 (44%), 105 (50%), 72 (100%).

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