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TRITERPENOID CONSTITUENTS OF *BUXUS PAPILLOSA*

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Abstract—Phytochemical investigations on the leaves of *Buxus papillosa* have yielded a new steroidal base (−)-cyclobuxoviramine The roots of the plant have afforded an unusual cycloartenol-type triterpenoid, (−)-buxatenone The structures were established on the basis of 2D NMR and other spectroscopic studies

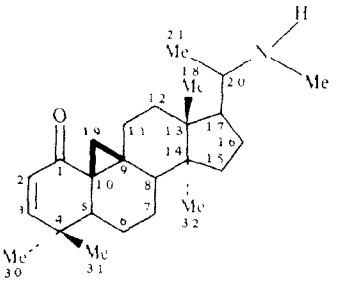
INTRODUCTION

The leaves of *Buxus papillosa* C K Schneider, family Buxaceae, find extensive use in folk medicine for the treatment of malaria, rheumatism and skin diseases Previous investigations on the plant have been on its aerial parts and have resulted in the isolation and characterization of over 40 new steroidal alkaloids by our group in Pakistan [1–8]. We describe here the isolation of a new steroidal base, (−)-cyclobuxoviramine (**1**) from the leaves of the plant This paper also describes the first phytochemical investigations on the roots of *Buxus papillosa* which have afforded a new triterpenoid, (−)-buxatenone (**2**), which may be a biosynthetic precursor to (−)-cyclobuxoviricine [8] and other related steroidal bases

RESULTS AND DISCUSSION

Our first compound, (−)-cyclobuxoviramine, afforded the formula $C_{25}H_{39}NO$ by high resolution mass measurement, indicating the presence of seven double bond equivalents in the molecule. The UV spectrum showed a strong absorption at 269 nm, characteristic of an α,β-unsaturated ketone conjugated with the cyclopropyl ring [7, 8] The IR spectrum displayed an intense absorption at 1675 cm^{-1} in agreement with the presence of an α,β-unsaturated carbonyl function Other absorptions were at 3350 (N–H) and 1600 (C=C) cm^{-1}

The ^1H NMR spectrum of the compound revealed its close resemblance to (−)-cyclobuxoviricine [8] It included four three-proton singlets at δ0 88, 0.90, 1 04 and 1 05 assigned to the four tertiary methyl groups A three-proton doublet centred at δ1 16 (J = 6 1 Hz) was due to the C-21 secondary methyl protons. A doublet at δ0 78 (J = 4 0 Hz) was assigned to the C-19α proton, the other half of the AB doublet being apparently embedded in the methyl/methylenic region of the spectrum, due to the electron withdrawing effect of the C-1 carbonyl group [7, 8]. A singlet at δ2 47 was due to the N-Me group The chemical shift of the N-Me signal indicated its presence at C-20 of the ring D side chain Two AB doublets at δ5 88 and 6 35 (J = 10 1 Hz) were assigned to the C-2 and C-3 olefinic protons, respectively, the chemical shifts indicating that the protons were part of an α,β-unsaturated ketone system The overall ^1H NMR spectrum of (**1**) distinctly resembled that of (−)-cyclobuxoviricine, the



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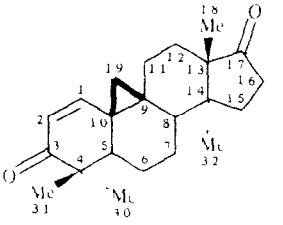

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major difference being in the olefinic region of the two compounds (−)-Cyclobuxoviricine, which has a C-1/C-2 double bond, has the resonances of the olefinic protons at δ5 94 and 6 73 ($J_{1,2}$ = 10 0 Hz) The upfield shift of the olefinic protons in compound **1** as compared to cyclobuxoviricine is consistent with its location away from deshielding influence of the cyclopropyl ring Since other spectral properties were similar, the two compounds appeared to be geometrical isomers

The ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) showed signals at δ14 74, 20 46, 16 01 and 23 10 which were assigned to the four tertiary methyl carbons Three downfield signals at δ124 70, 155 61 and 204 76 were assigned to the olefinic carbons C-2 and C-3, and to the carbonyl group at C-1, respectively The multiplicities of the various carbons were ascertained by DEPT and GASPE experiments and are presented in Table 1

The mass spectrum of compound **1** showed the molecular ion at m/z 369 3024 in agreement with the molecular

Table 1. ^{13}C NMR chemical shifts of cyclobuxoviramine (1)

C	Chemical shift (δ)	C	Chemical shift (δ)	C	Chemical shift (δ)
1	204 76	10	32 66	19	20 46
2	124 68	11	26 97	20	58.58
3	155 93	12	33 06	21	17 30
4	45 60	13	45 03	30	23 10
5	40.15	14	49 31	31	16 01
6	27.01	15	31 69	32	21 38
7	29.25	16	29 45	N–Me	28 40
8	40.28	17	47 90		
9	25 41	18	14 74		

formula, $C_{25}H_{39}NO$ (calcd 369.3031). A peak at *m/z* 354 resulted from the loss of methyl group from the molecular ion Another peak at *m/z* 339 corresponded to the loss of-NHMe group from the molecular ion. The base peak at *m/z* 58 was due to dimethyliminium ion, $MeCH=N^+H$-Me, resulting from the cleavage of mono-methyl amino group-containing ring D side chain [9]. This confirmed the presence of a mono-methyl amino group at C-20. The above studies led to structure **1** for this new steroidal base, named as (−)-cyclobuxoviramine. It may arise by the reduction of (−)-cyclobuxoviricine to the corresponding allylic alcohol followed by an allylic rearrangement.

Our second substance, (−)-buxatenone (**2**), was found to be a degraded cycloartenol triterpenoidal compound. Its molecular formula, confirmed by high resolution measurements, is $C_{22}H_{30}O_2$, indicating the presence of eight double bond equivalents in the molecule. It showed UV absorption at 265 nm as in (−)-cyclobuxoviricine [8] indicating an α,β-unsaturated ketonic function at ring A of the triterpenoidal skeleton. The IR spectrum includes absorptions at 1730 (five-membered ketonic carbonyl [10]), 1660 (α,β-unsaturated carbonyl) and 1610 (C=C) cm^{-1}.

The ^1H NMR spectrum of compound **2** showed four three-proton singlets resonating at δ0.83, 0.95, 1.08 and 1.12, which were assigned to C-32, C-30, C-18 and C-31 methyl protons respectively. Two AB doublets at δ0.68 and 1.38 ($J=4.9$ Hz) were due to the C-19α and C-19β cyclopropyl protons. The downfield shift of the C-19β methylenic proton from the usual values (δ0 1–0 5) was due to the electron withdrawing effect of the conjugated double bond The ^1H NMR spectrum of **2** interestingly showed four downfield double doublets at δ1.65, 1.78, 2.17 and 2.44, which were assigned to H-15α, H-15β, H-16α, and H-16β, respectively. The downfield shift of the last two signals was due to their α-disposition to the carbonyl group. The ^1H NMR spectrum showed lack of any signal for N-CH_3, 21-CH_3 and H-20, which suggested the absence of any side chain at ring D. The 2D ^1H NMR experiments (COSY-45, NOESY) [11] confirmed the above mentioned assignments for various protons. The COSY interactions between H-15α, H-15β, H-16α and H-

Table 2. ^{13}C NMR, multiplicities (DEPT) and ^1H–^{13}C connectivities (heteroCOSY)

C	Chemical shift (δ)	Multiplicity	^1H–^{13}C connectivity (δ)
1	152.58	CH	6.73
2	127 28	CH	5.94
3	204.69	–C–	—
4	45 79	–C–	–
5	43 69	CH	2 10
6	18 72	CH_2	*
7	24 57	CH_2	*
8	40 54	CH	2 17
9	25 26	–C–	—
10	44.08	–C–	—
11	21 29	CH_2	*
12	26 61	CH_2	*
13	52.72	–C–	—
14	30 13	–C–	—
15	30 82	CH_2	1 65 (H-15α), 1 78 (H-15β)
16	33 74	CH_2	2 17 (H-16α), 2 44 (H-16β)
17	220 12	–C–	—
18	21.44	CH_3	1 08
19	27 03	CH_2	0 68 (H-19α), 1 37 (H-19β)
30	19 21	CH_3	0 95
31	18 38	CH_3	1 12
32	19 21	CH_3	0.83

*Cross peak too weak to be detected in the heteroCOSY spectrum

16β further confirmed the unsubstituted nature of these two sites and the lack of any other proton at C-17 COSY interaction between the C-1 and C-2 olefinic protons was also observed.

The NOESY spectrum established the relative positions of different substituents in the molecule H-19α gave NOE interaction with H-19β as well as with H-1. Similarly H-1/H-2 and H-16α/H-16β were found to give NOE interactions.

The ^{13}C NMR chemical shifts of various carbons (multiplicities confirmed by DEPT) are presented in Table 2. Further confirmation of the structure comes from the heteroCOSY experiment The direct (one-bond) ^{1}H–^{13}C shift correlation (heteroCOSY) spectrum established that the C-19 methylenic carbon resonating at δ27.03 showed connectivity with H-19α (δ0.68) and with the deshielded H-19β (δ1 37) which was embedded in the methylenic envelope The heteroCOSY spectrum was also helpful in assigning the C-5 and C-8 methine protons at δ2 10 and 2 17. The ^{1}H–^{13}C correlations obtained are presented in Table 2

The mass spectrum of the compound **2** showed the molecular ion at *m/z* 326 2238 in agreement with the molecular formula $C_{22}H_{30}O_2$ (calcd 326.2245). A peak at *m/z* 311 arose by the loss of methyl group from the molecular ion. The compound showed the base peak at *m/z* 137 corresponding to the cleavage of ring B. The mass spectrum also showed the lack of any peak at *m/z* 58 which arises from the N-containing ring D side chain This observation along with biogenetic considerations and the studies mentioned above indicated C-17 as the site for the second carbonyl function Structure **2** was assigned for (−)-buxatenone on the basis of the above studies

EXPERIMENTAL

Mass spectra were recorded on a double focussing spectrometer connected to a computer system ^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz, while ^{13}C NMR spectra were also recorded in $CDCl_3$ at 100 MHz The purity of the samples was checked on TLC (silica gel, precoated plates) The plant material was identified by the plant taxonomist at the Department of Botany, University of Karachi, where a voucher specimen is deposited

Isolation of (−)-*cyclobuxoviramine* (**1**) An EtOH extract of *Buxus papillosa* (50 kg) collected from the northern regions of Pakistan (August, 1986), was evapd to a gum The total alkaloids were obtained by extraction into 10% AcOH. Partial separation of the alkaloids was carried out by extraction into $CHCl_3$ at different pH values The fraction obtained at pH 3 5 was loaded on a silica gel column (70–230 mesh ASTM, Merck) Elution with $CHCl_3$–MeOH (19 1) afforded a number of close-moving alkaloids The mixture was subjected to prep TLC (silica gel) employing hexane–Et_2NH (19 1) to afford compound **1** (10 3 mg), $[\alpha]_D^{20} = -35^\circ$

Isolation of (−)-*Buxatenone* (**2**) The EtOH extract of *Buxus papillosa* roots (100 kg) collected from the northern regions of Pakistan, was evapd to a gum The fractions were extracted with $CHCl_3$ at different pH values The fraction obtained at pH 3 5 was loaded on a silica column (70–230 mesh, ASTM, Merck) (−)-Buxatenone was eluted from the column along with other known compounds, and purified (23 8 mg) on TLC (silica gel) using the solvent system $CHCl_3$–$NHEt_2$ (19 1) $[\alpha]_D^{20} = -11^\circ$

REFERENCES

1 Atta-ur-Rahman, Choudhary, M I and Nisa, M (1985) *Heterocyles* **23**, 1951

2 Choudhary, M I, Atta-ur-Rahman, Freyer, A J. and Shamma, M (1986) *Tetrahedron* **42**, 5747

3 Choudhary, M I, Atta-ur-Rahman, Freyer, A J and Shamma, M (1987) *J Nat Prod* **50**, 84

4 Atta-ur-Rahman, Choudhary, M I and Nisa M (1987) *Planta med* 75

5 Atta-ur-Rahman and Choudhary, M I (1986), *J Chem Soc Perkin Trans, I* 919

6 Atta-ur-Rahman, Alam, M, Choudhary, M I and Firdous, S (1987), *Planta Med* **53**, 496.

7 Atta-ur-Rahman, Choudhary, M I, Ali, I. and Habib-ur-Rehman (1985) *J Nat Prod* **49**, 106

8 Atta-ur-Rahman, Choudhary, M I and Nisa, M (1985) *Phytochemistry* **24**, 3082

9 Waller, G R and Dermer, O C (1980) *Biochemical Applications of Mass Spectrometry* p 779 Wiley-Interscience, New York

10 Pavia, D L, Lampman, G M and Kriz, G S (1979) *Introduction to Spectroscopy* p 48 Saunders College, Philadelphia

11 Atta-ur-Rahman (1986) *Nuclear Magnetic Resonance Spectroscopy*, p 202 Springer, New York