30-Acetoxy-N^a-benzoylbuxidienine—A New Alkaloid from the Leaves of *Buxus papilosa*

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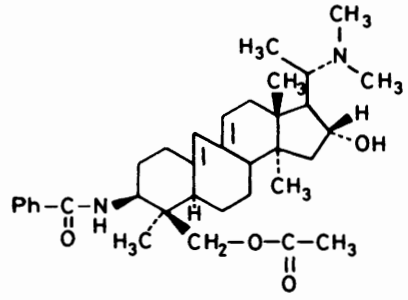
A new alkaloid 30-acetoxy-N^a-benzoylbuxidienine, to which structure (**1**) has been assigned, has been isolated from the leaves of *Buxus papilosa*.

Buxus papilosa, C.K. Schn. Linn (Buxaceae) is a shrub widely distributed in the northern regions of Pakistan. The extracts of the shrub have long been used in the indigenous system of medicine for the treatment of various ailments, including malaria, rheumatism, and skin diseases. In previous publications we have reported the isolation of several new alkaloids from the leaves of *Buxus papilosa*.[1-3] This paper describes the isolation and structure of a new steroidal alkaloid, to which structure (**1**) has been assigned.



(**1**)

The crude alkaloids were isolated from the concentrated alcoholic extracts of the leaves of *B. papilosa* by extraction at different pH values. The fraction obtained at pH 3.5 was loaded on a silica column and elution with 90% chloroform–10% methanol to afford a number of closely moving alkaloids. This mixture was subjected to preparative t.l.c. (p.l.c.) on silica plates with hexane–ethyl acetate–diethylamine (8.5:1.3:0.2) as eluant to afford compound (**1**) as a white amorphous material, $[\alpha]_D^{20}$ −4° ($CHCl_3$).

The i.r. spectrum of the substance showed intense absorptions at 3 400 (O–H), 3 350 (N–H), 1 716 (ester C=O), 1 662 (unsaturated amide C=O), and 1 610 cm^{-1} (C=C). The u.v. spectrum showed maxima at 238 (log ε 4.31), 245 (4.29), 253 (4.17), 268 (3.92), 279 (3.89), and 290 nm (3.87) with a shoulder at 205 nm, indicating the presence of a 9(10→19)*abeo*-diene system[1,2,4] and an aromatic ring; the latter was indicated from the ε value of the peak at 238 nm.

The high-resolution mass spectrum of the alkaloid showed the molecular ion at *m/z* 562.380 corresponding to the formula $C_{35}H_{50}N_2O_4$, indicating the presence of twelve double-bond equivalents in the molecule. The substance showed a peak at *m/z* 503.360 in accord with the composition $C_{33}H_{47}N_2O_2$ (**2**) resulting from the loss of an acetate group from the molecular ion. The loss of $OCOCH_3$ [59 mass units (m.u.)] rather than CH_3CO_2H (60 m.u.) suggested that the acetate was attached to a carbon with no α-H atoms. An important fragment at *m/z* 457.340 (**3**), having the composition $C_{28}H_{45}N_2O_3$, was consistent with the loss of a benzoyl group. Another peak at *m/z* 115.097, having the formula $C_6H_{13}NO$ (**4**), resulted from the cleavage of ring D along with the nitrogen-containing side-chain. The peak at *m/z* 85.087, with the composition $C_5H_{11}N^+$ (**5**), was attributed to the cleavage of ring D.[2,3] The peak at *m/z* 71.071, having the formula $C_4H_9N^+$, was assigned to fragment (**6**) formed by cleavage of the ring D side-chain.[1,2] The fragment at *m/z* 72.081, having the composition $C_4H_{10}N^+$ (**7**) $[CH_3CH{=}N^+(CH_3)_2]$, is a commonly encountered ion formed by the cleavage of the ring D side-chain.[5] The substance showed a base peak at *m/z* 58.065, corresponding to the composition $C_3H_8N^+$, which was assigned to the fragment (**8**), another commonly encountered fragment in other related alkaloids.[1,2] The key fragmentation processes are presented in Figure 1.

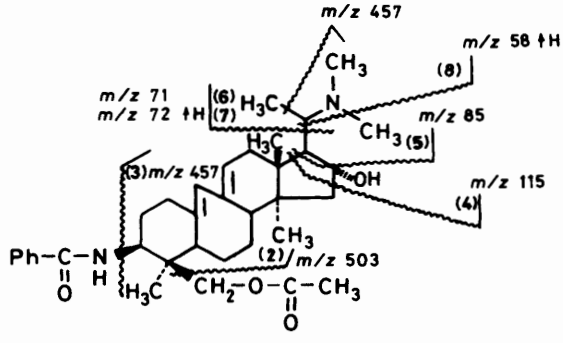


Figure 1. Postulated key mass-spectral fragmentations of compound (**1**)

The mass spectrum of compound (**1**), when recorded after dissolution in CD_3OD, showed a 2 m.u. increase in the molecular weight, indicating the presence of two exchangeable protons in the compound.

The ^1H n.m.r. spectrum ($CDCl_3$; 300 MHz) showed three tertiary methyl groups at δ_H 0.76, 0.77, and 0.94, while the secondary methyl group resonated as a doublet at δ_H 0.93 ($J_{21,20}$ 6.39 Hz). A 6 H singlet at δ_H 2.61 was assigned to the two methyl protons of the $N(CH_3)_2$ groups. Another 3 H singlet at δ_H 2.12 was assigned to the acetate methyl protons. The C-30 and methylene protons appeared as a set of doublets centred at δ_H 3.82 and 4.02 ($J_{30\alpha,30\beta}$ 10.98 Hz). A multiplet centred at δ_H 3.94 was assigned to the C-16 proton. A singlet at δ_H 6.07 was ascribed to the olefinic proton at C-19, while a multiplet at δ_H 5.52 was assigned to the C-11 olefinic proton.[6] The aromatic protons appeared as two groups of 3 H and 2 H multiplets centred at δ_H 7.43 and 7.71 respectively.

Two-dimensional n.m.r. measurements (2D-*J*-resolved,[7] NOESY,[8] and COSY-45[9,10]) fully agreed with the proposed structure (**1**) for 30-acetoxy-N^a-benzoylbuxidienine. The multiplicities of the proton signals were determined from the 2D-*J*-resolved spectrum, while the COSY-45 spectrum established the

coupling interactions. The NOESY spectrum served to show the relative stereochemistry at several key points in the molecule. Strong cross-peaks were observed corresponding to the C-11 and C-9 olefinic protons. The N^b–CH_3 signal showed n.O.e. interaction with the aromatic region (phenyl 4′-H). This interaction requires ring A to be in a twist-boat conformation. The n.O.e. interaction between the vicinal phenyl protons as well as between the C-19 olefinic proton and C-1β methylenic proton at δ_H 2.25 was also observed. The n.O.e. interaction between the N^b methyl protons and the C-18 methyl group established the β-orientation of the C-18 methyl group. Similarly, n.O.e. interaction between the C-29 methyl protons at δ_H 0.94 could be seen with the C-6 methylene proton at δ_H 1.91 which established the α-orientation of the C-29 methyl group. This showed that the OAc group was attached to the β-orientated C-30 methylene group. The NOESY interactions are shown in Figure 2.

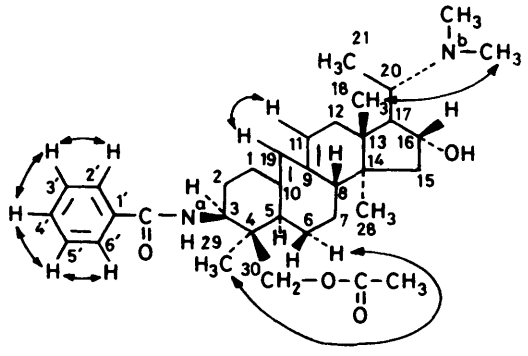


Figure 2. NOESY interactions of compound (1)

The ^{13}C spectrum in $CDCl_3$ showed four signals, at δ_C 10.29, 11.37, 16.61, and 18.40, which were assigned to C-18, C-28, C-29, and C-21 methyl carbons.[11] The C-19 olefinic carbon appeared at δ_C 126.92, while the C-11 olefinic carbon resonated at δ_C 125.92. The methyl of the acetyl group resonated at δ_C 21.00 whereas the C-30 carbon appeared at δ_C 65.36.[11] The assignments of the signals to carbon atoms are shown in the Table.

Experimental

Mass spectra were recorded on a Varian MAT 312 double focussing spectrometer connected to a PDP 11/34 computer system. The ^1H n.m.r. spectra were recorded in $CDCl_3$ solution on a Bruker AM-300 NMR spectrometer at 300 MHz, while the ^{13}C n.m.r. spectra were recorded at 75 MHz; n.m.r. peaks are recorded as δ values from internal $SiMe_4$ as reference. The i.r. spectrum was recorded on a Jasco IRA-1 infrared spectrometer. The u.v. spectrum was recorded on a Shimadzu UV 240 instrument. The optical rotation was taken on a Polartronic D polarimeter. The purity of samples was checked on t.l.c. (silica gel, SiF, precoated plates).

Extraction and Isolation of compound (1).—Three ethanolic extracts (190 l) of *Buxus papilosa* leaves (50 kg) collected from the northern regions of Pakistan were combined and evaporated to a gum. The total alkaloids (500 g) were obtained by extraction into 10% CH_3CO_2H.* Partial separation of the alkaloids was carried out by extraction into $CHCl_3$ at different pH values. The fraction obtained at pH 3.5 (14 g) was loaded on a silica column (0.2—0.5 mm, 35—70 mesh ASTM). Elution with 90% $CHCl_3$–10% MeOH afforded a fraction (1.75 g) containing a number of closely moving alkaloids. The mixture was subjected to p.l.c. (silica gel) with hexane–ethyl acetate–diethylamine (8.5:1.3:0.2) as eluant, to afford 30-acetoxy-N^a-benzoylbuxidienine (**1**) as a white amorphous material (7.80 mg), $[\alpha]_D^{20}$ −4° (*c* 8.54 × 10^{-5} mol l^{-1} in $CHCl_3$); *m/z* 562.380 (M^+, 14%, $C_{35}H_{50}N_2O_4$, calc. for M^+, 562.377), 547.350 (4, $C_{34}H_{47}N_2O_4$, calc. 547.353), 503.360 (4, $C_{33}H_{47}N_2O_2$, calc. 503.363), 457.340 (9, $C_{28}H_{45}N_2O_3$, calc. 457.342), 115.097 (5, $C_6H_{13}NO$, calc. 115.099), 85.087 (62, $C_5H_{11}N$, calc. 85.089), 72.081 (92, $C_4H_{10}N$, calc. 72.081), 71.071 (40, C_4H_9N, calc. 71.073), and 58.065 (100, C_3H_8N, calc. 58.065); $\nu_{max.}$(MeOH) 3 400 (O–H), 3 350 (N–H), 1 716 (C=O ester), 1 662 (C=O amide), and 1 610 cm^{-1} (C=C); $\lambda_{max.}$($CHCl_3$) 238, 245, 253, 268, 279, and 290 nm (log ε 4.13, 4.29, 4.17, 3.92, 3.89, and 3.87); δ_H 0.76 (3 H, s, t-Me), 0.77 (3 H, s, t-Me), 0.93 (3 H, d, $J_{21,20}$ 6.39 Hz, 21-Me), 0.94 (3 H, s, t-Me), 2.12 (3 H, s, OCOMe), 2.61 (6 H, s, N^bMe_2), 3.82 (1 H, d, $J_{30\alpha,30\beta}$ 10.98 Hz, 30-H_α), 4.02 (1 H, d, $J_{30\beta,30\alpha}$ 10.98 Hz, 30-H_β), 3.94 (1 H, m, 16-H), 5.52 (1 H, m, 11-H), 6.07 (1 H, s, 19-H), and 7.43—7.71 (5 H, m, ArH); δ_C, see the Table.

Table. ^{13}C N.m.r. data for compound (1)[a]

Carbon	Chemical shift	Carbon	Chemical shift
1	36.00	18	61.61
2	30.22	19	126.92
3	47.28	20	60.30
4	43.90	21	10.29
5	50.76	N^b–CH_3	39.77
6	25.70	N^b–CH_3	42.11
7	29.50	28	18.40
8	49.89	29	11.37
9	138.70*	30	65.36
10	138.30*	1′	136.90
11	125.92	2′	128.67
12	32.00	3′	129.00**
13	45.37	4′	131.10***
14	48.60	5′	130.00***
15	42.90	6′	129.20**
16	77.80	PhC(=O)–N	167.25
17	63.30	C(=O)–CH_3	21.00
		C(=O)–CH_3	171.34

[a] Recorded in $CDCl_3$ at 75 MHz.
*,**,***, The values are interchangeable.

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* The extraction has been subsequently repeated with 5% HCl to ascertain that compound (**1**) is not an artefact formed by acetic acid extraction.

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