THE ISOLATION AND STRUCUTRE OF BUXAQUAMARINE - A NEW STEROIDAL ALKALOID FROM *BUXUS PAPILOSA*

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Abstract - A new steroidal alkaloid, buxaquamarine, has been isolated from the leaves of Buxus papilosa to which structure (I) has been assigned.

Busus papilosa (Buxaceae) is a shrub which occurs abundantly in the northern regions of Pakistan. The extracts of the plant have long been used in the indigenous system of medicine for the treatment of various ailments, including malaria and skin diseases. A number of alkaloids have previously been reported by us from this plant. [1-4] We now report the isolation and structure of a new alkaloid, buxaquamarine, from its leaves.

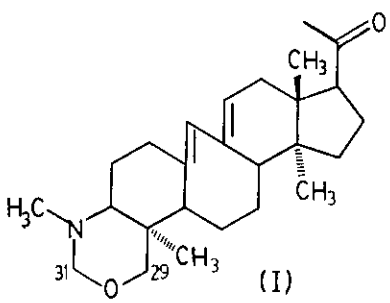
The crude alkaloids were isolated from the concentrated alcoholic extracts of the leaves of Buxus papilosa by extraction at different pH values. The fraction obtained at pH 3.5 was loaded on a silica gel column and elution with 90% chloroform-10% methanol afforded a number of close-moving alkaloids. This mixture was subjected to preparative t.l.c. on silica gel plates with hexane:acetone: diethylamine (9:9:2) to afford buxaquamarine as a white gummy material.

The ir spectrum of the substance showed intense bands at 1685, 2840 and 1645 cm^{-1}. The uv spectrum showed maxima at 238 and 245 nm and shoulders at 205 and 253 nm, characteristic of presence of the 9 (10→19) abeo-diene system.[5] An identical uv spectrum is encountered in moenjodaramine,[1] harappamine[2] and buxaminol E.[5] The proton NMR spectrum ($CDCl_3$) at 300 MHz showed a set of AB double doublets resonating at δ 3.27 and δ3.84 which were assigned to C-29 α and β methylene protons respectively ($J_{29\alpha,29\beta}$=10.66), while another set of AB double doublets at δ 3.61 and δ 4.45 ($J_{31\alpha,31\beta}$=7.56 Hz) was attributed to the methylene protons adjacent to the C-3 nitrogen.[1,2] Irradiation at δ 3.27 resulted in the collapse of the doublet at δ 3.84 into a sharp singlet. Similarly irradiation at δ 3.61 caused the doublet at δ4.45 to collapse to a sharp singlet. A singlet at δ6.00 was ascribed to the isolated olefinic proton at C-19 while a multiplet centered at δ5.60 was assigned to the C-11 olefinic proton. The spectrum showed signals due to three tertiary methyl groups at δ0.68, δ0.75 and δ1.06. A 3H singlet at δ 2.17 was assigned to a methyl group α to the carbonyl group. Another 3H

singlet at δ 2.12 was assigned to the $-NCH_3$ group.

The mass spectrum of buxaquamarine showed the molecular ion at m/z 397.2980 corresponding to the formula $C_{26}H_{39}NO_2$ (calcd. 397.2981). The substance showed a peak at m/z 382.7441 corresponding to the loss of methyl group from the M^+ ion.[6] A peak at m/z 354.2759 was in accordance with the composition $C_{24}H_{36}NO$ (calcd. 354.2796) (2) resulting from the loss of acetyl group from the molecular ion. An important fragment at m/z 127.1002 (3) having the composition $C_7H_{13}NO$ (calcd. 127.0997) may arise by cleavage of the ring A accompanied by an intramolecular proton transfer. Another peak at m/z 85.0699 (4) was in accordance with the composition $C_5H_{11}N$ (calcd. 85.0891) and was attributed to the cleavage of ring A along with the side chain. The peak at m/z 70.0658 (5) having the composition C_4H_8N (calcd. 70.0656) was consistent with a fragment $CH_2=CH-N^+(CH_3)=CH_2$ formed by the cleavage of ring A. Another peak at m/z 71.0856 having a formula C_4H_9N (calcd. 71.0734) is assigned to the fragment (6) formed by the cleavage of ring A along with the side chain accompanied by an intramolecular proton transfer. The peak at m/z 72.1311 (7) having the composition $C_4H_{10}N$ was consistent with a fragment $CH_3CH=N^+(CH_3)_2$ formed similarly by the cleavage of ring A and double hydrogen transfer†. The substance showed a base peak at m/z 58.0651 corresponding to the composition C_3H_8N which was attributed to the loss of fragment (8), a commonly encountered fragmentation in other related alkaloids.[1,2] Similarly a peak at m/z 57.0582 (9) having the composition C_3H_7N resulted from the cleavage of ring A. The key fragmentations are presented in scheme 1.

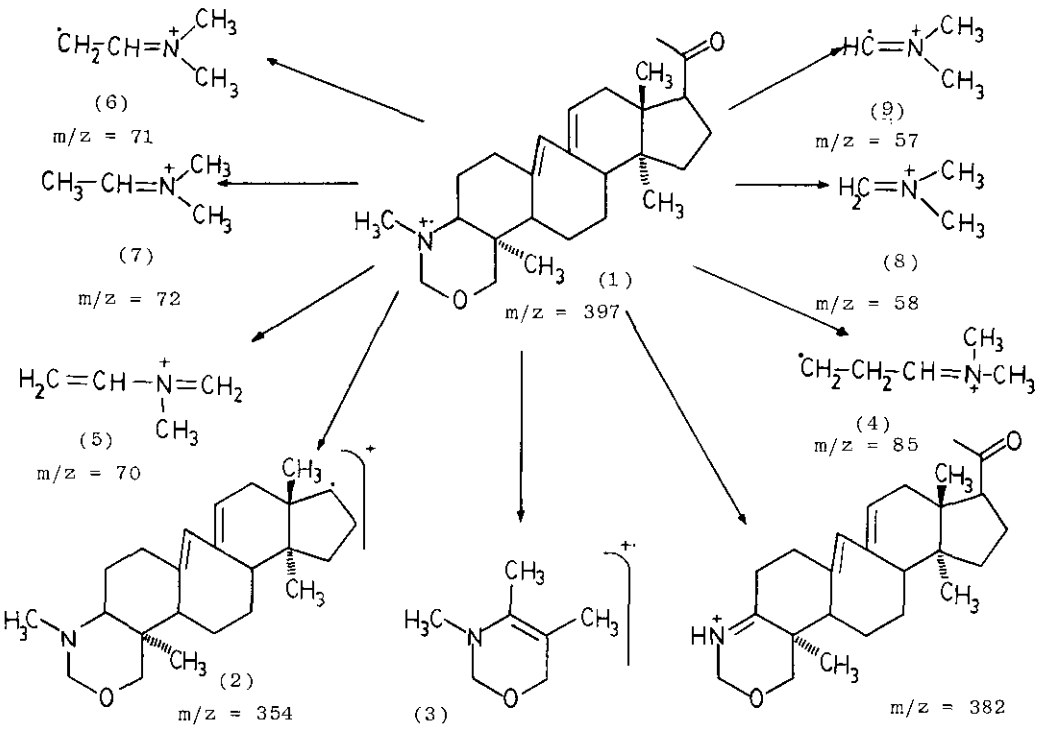
In the light of the above studies structure (1) is assigned to buxaquamarine. It may arise in nature from moenjodaramine or harappamine by oxidation of the N-bearing side chain to the corresponding ketimine, followed by its hydrolytic removal.



(I)

†In previous publications,[1,2] we have reported that the peak at m/z 71 arose by the cleavage of ring A while m/z 72 was formed by the cleavage of ring D side chain. The mass spectrum of buxaquamarine however indicates that the peak at m/z 72 could also arise by the cleavage of ring A and intramolecular double hydrogen transfer.

Scheme 1



EXPERIMENTAL

Mass spectra were recorded on a Varian MAT 312 double focussing spectrometer connected to PDP 11/34 computer system. The ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Bruker AM-300 NMR spectrometer at 300 MHz. Chemical shifts (δ) are expressed in ppm. Ir spectra were recorded on a Jasco IRA-1 infrared spectrophotometer. The uv spectra were recorded on a Shimadzu UV 240 instrument.

EXTRACTION AND SEPARATION - The ethanolic extract of the Buxus papilosa leaves (50 kg) collected from the northern regions of Pakistan, was evaporated to a gum. The total alkaloids were obtained by extraction in to 10% CH_3COOH. 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 gm) was loaded on a silica gel column(0.2-0.5 mm, 35-70 mesh ASTM). Elution with 90% $CHCl_3$-10% MeOH afforded a fraction (1.75 gm) containing a number of close-moving alkaloids. The mixture was subjected to prep. tlc. (silica gel) employing hexane-acetone-diethylamine (9:9:2), to afford buxaquamarine as a white gummy material (9.0 mg), $[\alpha]_D$= +24° ($CHCl_3$); ms, m/z 397.2980 (M^+, $C_{26}H_{39}NO_2$), 382.7441 ($C_{25}H_{36}NO_2$), 354.2759 ($C_{24}H_{36}NO$), 127.1002 ($C_7H_{13}NO$), 85.0699 ($C_5H_{11}N$), 72.1311 ($C_4H_{10}N$), 71.0856 (C_4H_9N), 70.0658 (C_4H_8N),

58.0651 (C_3H_8N), 57.0582 (C_3H_7N); ir ($CHCl_3$), 2840 (C-H), 1685 (-$\overset{O}{\overset{\|}{C}}$-), 1645 cm^{-1} (C=C); ^{1}H-NMR (δ,$CDCl_3$), 0.68 (3H, s, C$\underline{H}_3$), 0.75 (3H, s, C$\underline{H}_3$), 1.06 (3H, s, C$\underline{H}_3$), 2.12 (3H, s, N-C$\underline{H}_3$), 2.17 (3H, s, -$\overset{O}{\overset{\|}{C}}$-C$\underline{H}_3$), 3.27 (1H, dd, 29$\alpha$H, $J_{29\alpha,29\beta}$=10.66Hz), 3.61 (1H, dd, 31αH, $J_{31\alpha,31\beta}$=7.56Hz) 3.84 (1H, dd, 29βH, $J_{29\beta,29\alpha}$=10.66Hz), 4.45 (1H, dd, 31βH, $J_{31\beta,31\alpha}$=7.56 Hz), 5.60 (1H, bm, 11$\underline{H}$), 6.00 (1H, s, 19$\underline{H}$).

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