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PAPILLAMIDINE - A NEW STEROIDAL ALKALOID FROM THE LEAVES OF <u>BUXUS PAPILLOSA</u>

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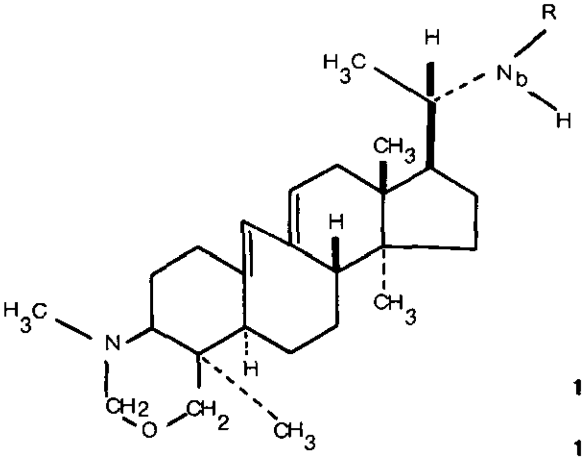
Abstract - A new steroidal alkaloid, papillamidine, has been isolated from the leaves of Buxus papillosa, to which structure **1** has been assigned.

Buxus papillosa C.K. Schneider (Buxaceae) is a shurb widely distributed in the northern region of Pakistan. In previous publications, we have reported the isolation of several new alkaloids from the leaves of B. papillosa[1-8]. This paper describes the isolation and structure of another new alkaloid, which contains a tetrahydrooxazine ring in its structure.

The crude alkaloids were isolated from the concentrated alcoholic extracts of leaves of B. papillosa by extraction at different pH values. The fraction obtained at pH 8.0 was evaporated to a gum. The column and thin-layer chromatographic separation of this fraction yielded amorphous papillamidine (**1**).



1 R = $-\overset{\overset{O}{\|}}{C}H$

1A R = CH_3

The uv spectrum of compound 1 showed absorption maxima at 238 and 246 and shoulders at 228 and 254 nm, characteristic of 9(10 → 19) abeo-diene system[8]. The ir spectrum displayed an intense absorption at 1665 cm^{-1} for the amide carbonyl; other absorptions were at 3600 (N-H) and 1596 (C=C) cm^{-1}.

The ^1H-nmr ($CDCl_3$, 400 MHz) spectrum bore a distinct similarity with that of (+)-harappamine (**1A**)[8]. It showed three tertiary methyl singlets at δ 0.67, 0.78 and 1.03. A doublet centered at δ 0.96 (J= 6.4 Hz) was assigned to the 21-secondary methyl group, while the neighbouring C-20 methine appeared as a multiplet at δ 2.98. A three-proton singlet at δ 2.11 was due to the N-methyl group. A set of AB doublets resonating at δ 3.24 and 3.81 (J= 10.6 Hz) were assigned to the C-31 methylenic protons while another set of AB doublets at δ 3.58 and 4.43 (J= 7.5 Hz) were due to C-33 methylenic protons. The C-11 vinylic proton appeared as a broad singlet at δ 5.53, while the isolated C-19 vinylic proton resonated as a singlet at δ 5.96. The N_b-formyl proton appeared as a doublet at δ 8.12, accompanied by a much smaller doublet at δ 8.13, representing the two non-separable rotamers.

Two dimensional ^1H-nmr experiments (2D J-resolved, COSY 45°, NOESY)[9,10] fully agreed with the proposed structure **1**. The multiplicities of overlapping proton signals could be determined from the 2D J-resolved spectrum while the COSY 45° spectrum established the coupling interactions among vicinal protons. The C-21 methyl protons showed cross peaks in the COSY spectrum with the C-20 proton at δ 2.98. The COSY spectrum also showed coupling interaction of the C-11 proton with the C-12 methylenic proton at δ 2.21.

The high resolution mass spectrum of compound **1** includes molecular ion at m/z 426.3229 corresponding to the molecular formula, $C_{27}H_{42}N_2O_2$ (calcd. 426.3246). A peak at m/z 411.3022 ($C_{26}H_{39}N_2O_2$, calcd. 411.3011) was due to the loss of methyl group from the molecular ion. A considerably large peak at m/z 127.0998 ($C_7H_{14}NO$, calcd. 127.1075) arose by the cleavage of ring A, characteristic of Buxus alkaloids bearing an unsubstituted tetrahydrooxazine ring[8]. The substance showed a base peak at m/z 100.0746 ($C_5H_{10}NO$, calcd. 100.0762) due to the cleavage of ring D along with N-formyl containing side chain. A large peak at m/z 72.0455 (C_4H_8N, 72.0449) representing N-formylmethyliminium cation, $CH_3\text{-}CH{=}N^{+}H\overset{O}{\overset{\|}{C}}H$ arose by the cleavage of the ring D side chain. Other peaks were at m/z 71, 58 and 57.

The overall spectral image of compound **1** resembled (+)-harappamine (**1A**) and infact species **1** corresponds to the N-formyl-N_b-norderivative of (+)-harappamine.

EXPERIMENTAL

The leaves of B. papillosa were collected in northern Pakistan by the Forest Institute, Peshawar. The plant was identified by Professor S. Irtifaq Ali, Department of Botany, University of Karachi, and a specimen has been deposited in the Department of Botany, University of Karachi.

The EtOH extract of the air-dried leaves (50 kg) of B. papillosa was evaporated under vacuum to afford a gum. This was taken up in 10% AcOH. The aqueous acidic extract was basified with NH_4OH and extracted with $CHCl_3$. The crude alkaloids (75 g) obtained upon evaporation of the organic solvent were loaded on a silica gel column (3.2 kg). Elution was with $CHCl_3$-MeOH mixtures of increasing polarity. The fraction obtained using $CHCl_3$-MeOH (6:4) weighed 2 .38 g. This fraction was placed on a silica gel column (200 g) and eluted with $CHCl_3$-MeOH (9:1). The main fractions were subjected to repeated prep. TLC on silica gel, using the system $CHCl_3$ - MeOH (9.8:0.2) to afford amorphous 1, $[\alpha]_D = 0^o$ ($CHCl_3$), uv λ_{max} (nm): 228,238,246 and 254; ir ν_{max} (cm^{-1}): 3600 (N-H), 1665 (amide carbonyl) and 1595 (C=C); ^{1}H-nmr ($CDCl_3$): δ 0.67 (3H, s, $C\underline{H}_3$), 0.78 (3H,s, $C\underline{H}_3$), 0.96 (3H, d, J= 6.4 Hz, 21-$C\underline{H}_3$), 1.03 (3H, s, $C\underline{H}_3$), 2.11 (3H, s, N_a-$C\underline{H}_3$), 2.98 (1H, m, 20-$\underline{H}$), 3.24 (1H, d, J=10.6 Hz, 31$\alpha\underline{H}$), 3.58 (1H, d, J=7.5 Hz, 33$\alpha\underline{H}$), 3.81 (1H, d, J=10.6 Hz, 31$\beta\underline{H}$), 4.43 (1H, d, J= 10.6 Hz, 33$\beta\underline{H}$), 5.53 (1H, bs, 11-H) and 5.96 (1H, s, 19-$\underline{H}$); high-ms m/z (rel.Int. %), 426.3229 (M^+, calcd. for $C_{27}H_{42}N_2O_2$ 426.3246), (52), 411.3022 ($C_{26}H_{39}N_2O_2$) (42), 127.0998 ($C_7H_{13}NO$) (92), 100.0746 ($C_5H_{10}NO$) (100), 72.0455 (C_3H_6NO) (61), 58.0579 (C_3H_8N) (87), 57.0562 (C_3H_7N) (22).

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