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(+)-*N*-FORMYLHARAPPAMINE AND (+)-*N*-FORMYLPAPILICINE, TWO NEW STEROIDAL ALKALOIDS FROM *BUXUS PAPILOSA*

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Key Word Index—*Buxus papilosa*, Buxaceae, steroidal alkaloids; (+)-*N*-formylharappamine, (+)-*N*-formylpapilicine

Abstract—Two new steroidal alkaloids from *Buxus papilosa* C.K. Schneider (Buxaceae) of Pakistani origin are (+)-*N*-formylharappamine (**1**) and (+)-*N*-formylpapilicine (**2**).

INTRODUCTION

Buxus papilosa C. K. Schneider (Buxaceae) is a shrub native to northern Pakistan. Our continuing studies on this plant [1–4] have now resulted in the isolation of two new alkaloids, namely (+)-*N*-formylharappamine (**1**) and (+)-*N*-formylpapilicine (**2**).

RESULTS AND DISCUSSION

The crude alkaloids were isolated from the air dried leaves of *B. papilosa* as described previously [3, 4] The $CHCl_3$ extract, obtained by extraction at pH 8.5, was concentrated and subjected to column chromatography Further purification by preparative TLC resulted in the isolation of compounds **1** and **2**.

(+)-*N*-Formylharappamine (**1**), $C_{28}H_{44}N_2O_2$, showed UV maxima at 238 and 245 nm, with shoulders at 225 and 254 nm This absorption pattern is characteristic of a 9(10 → 19)-*abeo*diene system [2]. The IR spectrum featured strong absorptions at 1653 (amide) and 1624 (C=C) cm^{-1}.

The ^1H NMR spectrum ($CDCl_3$, 360 MHz) of **1** bore a distinct similarity to that of (+)-harappamine (**1A**) [2], and included three singlets, at δ0.70, 1.09 and 1.20 The secondary methyl groups absorbed as a doublet at δ0.83. A three-proton singlet located at δ2.20 was assignable to the N_a-methyl group. A set of AB doublets resonating at δ3.25 and 3.84 represented the C-30 methylene protons, while another set of AB doublets centered at δ3.60 and 4.48 indicated the protons of the methylene group bridging the nitrogen and oxygen atoms in the tetrahydrooxazine ring A singlet at δ5.98 and a doublet of doublets centered at δ5.56 were ascribed to H-19 and H-11, respectively. A singlet at δ8.11, accompanied by a much smaller singlet at 7.98, represented the N_b-formyl proton. Similarly, the N_b-methyl group was indicated by a singlet at δ2.74, followed by a smaller singlet at 2.80, due to geometrical isomerism.

The mass spectrum of (+)-*N*-formylharappamine (**1**) displayed molecular ion *m/z* 440. Base peak *m/z* 86 represented the *N*-formyldimethyliminium cation, $[Me–CH=N(Me)CHO]^+$ Fragment *m/z* 127 arose through cleavage of ring A accompanied by proton transfer.

Our second alkaloid, (+)-*N*-formylpapilicine (**2**), $C_{28}H_{46}N_2O$, exhibited UV absorption maxima at 238 and 245 nm, with shoulders at 225 and 255 nm, again diagnostic of the 9(10 → 19)-*abeo*diene system. The IR spectrum showed intense bands at 1658 (amide) and 1599 (C=C) cm^{-1}

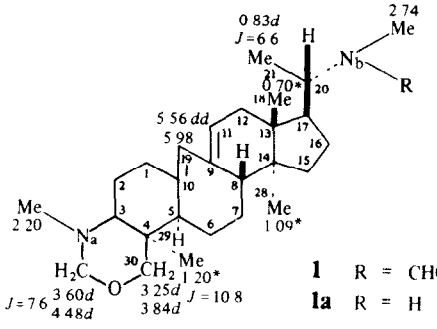
The ^1H NMR spectrum was closely related to that of the known (+)-papilicine (**2A**) [1]. It included four three-proton singlets at δ0.70, 0.80, 0.81 and 1.14, indicating the number of tertiary methyl groups present. The secondary (C-21) methyl group resonated as a doublet at δ1.20. A six-proton singlet at δ2.43 was assigned to the $N(Me)_2$ group attached to C-3. The C-11 olefinic proton appeared as a doublet of doublets at δ5.53, while the C-19 olefinic proton absorbed as a singlet at δ5.93. As with compound **1**, the formyl proton appeared as a singlet at δ8.11 (and 7.99), and the N_b-methyl resonated at δ2.74 (and 2.80)

The mass spectrum of (+)-*N*-formylpapilicine (**2**) showed molecular ion *m/z* 426 Peak *m/z* 383 resulted from loss of the methyliminium moiety from ring A. Fairly large ion *m/z* 86 represented the $[Me–CH=N(Me)CHO]^+$ fragment Finally, peak *m/z* 71 derived from cleavage of ring A as indicated in expression **2**.

In addition to the aforementioned steroidal bases, the plant yielded (+)-sinapic acid methyl ester (**3**), $C_{12}H_{14}O_5$. This compound incorporates the E configuration around the side chain double bond. ^1H NMR chemical shift assignments have been indicated around expression **3** [5–7].

EXPERIMENTAL

The leaves of *B papilosa* 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



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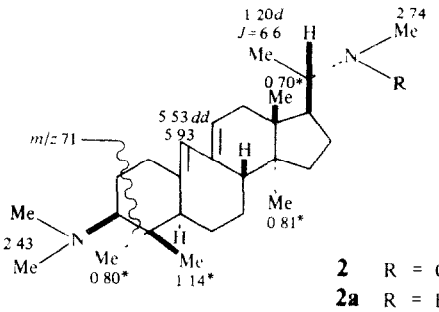
1a R = H

H-11 J_1 = 2.4 Hz
J_2 = 1 6 Hz



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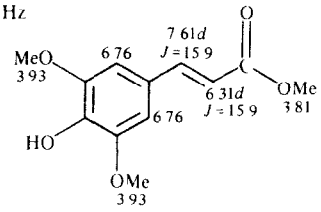

3

The EtOH extract of the air-dried leaves (50 kg) of *B papilosa* was evaporated under vacuum to afford a gum This was taken up in 10% HOAc The aq acidic extract was basified with NH_4OH and extracted with $CHCl_3$ The crude alkaloids (75 g) obtained upon evapn 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(93 7) weighed 2 74 g This fraction was placed on a silica gel column (130 g), and eluted with C_6H_{14}–$CHCl_3$–Et_2NH (14 5 1) The main fractions were subjected to repeated prep TLC on silica gel, using the system C_6H_{14}–Me_2CO–Et_2NH (8 1 1) to afford compounds **1**–**3**

(+)-N-*Formylharappamine* (**1**) Amorphous (6 mg), $[\alpha]_D$ = +40° ($CHCl_3$, *c* 0 50), UV λ_{max}^{MeOH} nm (log ε) 225, 238, 245, 254 (3 96, 4 04, 4 03, 3 90), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1653, 1624, MS *m/z* (rel int) 440 (M^+, 70), 425 (30), 411 (10), 127 (35), 86 (100), 71 (40), 58 (90), 44 (40), 28 (38)

(+)-N-*Formylpapilicine* (**2**) Amorphous (4 mg); $[\alpha]_D$ = +36° ($CHCl_3$, *c* 0 35), UV λ_{max}^{MeOH} nm (log ε) 225, 238, 245, 255 (4 00, 4 20, 4 23, 4 04), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1658, 1599, MS *m/z* (rel int) 426 (M^+, 11), 411 (2), 397 (2), 383 (14), 86 (32), 85 (30), 71 (100), 58 (50)

(+)-*Sinapic acid methyl ester* (**3**) Amorphous (2 mg), $[\alpha]_D$ = +16 (*c* 1 23, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε) 239, 327 (3 62, 3 57), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1699, 1603, MS *m/z* (rel int) 238 (M^+, 100), 223 (10), 175 (30), 28 (50)

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