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(+)-*N*-ACETYL-*N*-DEMETHYLCYCLOMICROBUXEINE—ISOLATION, STRUCTURE AND CONFORMATIONAL STUDIES

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Key Word Index—*Buxus papillosa*; Buxaceae; (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine; temperature-dependent ^{1}H NMR studies.

Abstract—The isolation and structure elucidation of a new steroidal alkaloid, (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine, from the leaves of *B. papillosa* is reported. Temperature-dependent studies of the ^{1}H NMR spectrum of (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine indicates that it exists as a mixture of two conformers at room temperature, which equilibrate at higher temperatures.

INTRODUCTION

Buxus papillosa C. K. Schneider (Buxaceae is a commonly grown shrub found in the northern regions of Pakistan. A number of steroidal alkaloids have been previously reported from this plant [1–5]. The present report describes the isolation, structure elucidation and conformational studies on a new steroidal alkaloid, (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine (**1**), from the leaves of *B. papillosa*.

RESULTS AND DISCUSSION

The ethanolic extract of *B. papillosa* leaves collected from the northern regions of Pakistan, was evapd as described in the Experimental section and partial separation of the alkaloids was carried out by extraction into chloroform at different pH values. The fraction obtained at pH 3.5 was loaded on a silica gel column. Elution was carried out with chloroform–methanol. Further purification of an important fraction was effected by TLC on silica gel to obtain (+)-*N*-acetyl-*N*-demethyl-cyclomicrobuxeine (**1**).

The UV spectrum of compound **1** showed a strong absorption maxima at 237 and 245 nm, indicating the presence of amide and α,β-unsaturated carbonyl groups, respectively. The IR spectrum displayed absorptions at 1645 cm^{-1} representing amide and α,β-unsaturated carbonyl groups, while the band at 1595 cm^{-1} was due to C=C stretching vibrations.

The mass spectrum of the compound showed a molecular ion at *m/z* 395.2910, corresponding to the molecular formula $C_{26}H_{37}NO_2$ (calcd 395.2824), indicating the presence of nine double bond equivalents in the molecule. A peak at *m/z* 380 was due to the loss of methyl group from the molecular ion. The compound showed a base peak at *m/z* 74.0604 (C_3H_8NO, calcd 74.0605), representing an amidic side chain of the ring A. The key fragmentations are presented in Fig. 1.

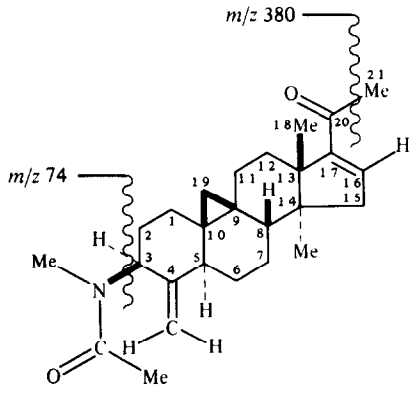
The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine (**1**) showed two three-proton doublets at δ0.99 and 1.19 indicating the presence of two tertiary methyl groups. Another three-proton singlet at δ2.26 was due to the C-21 methyl group. A close doublet of doublets at δ6.65 ($J_1 = 3.4$ Hz, $J_2 = 2.1$ Hz) was assigned to the C-16 olefinic protons. The unusually low coupling constant of this signal may be accounted for by the preferred *gauche* conformation of the olefinic proton with respect to the C-15 methylenic protons [5, 6]. The rest of the ^{1}H NMR spectrum displayed a doubling of many peaks (each integrating for half of the actual integration). Thus two AB doublets at δ0.14/0.41 and 0.11/0.38 were ascribed to cyclopropyl protons of the two conformers. Similarly doubling of the N_a-Me signal (δ2.88 and 2.91), N_a-acetyl methyl (δ2.14 and 2.05) were also observed. The exomethylenic protons also showed doubling of the singlets in the ^{1}H NMR spectrum of the compound at δ4.37/4.70 and 4.43/4.58.

Since the compound was pure, it was suspected that (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine (**1**) existed in a mobile equilibrium between the two amide conformers. The ^{1}H NMR spectrum was therefore recorded at increasing temperatures (20, 30, 40, 45, 50, 60, 70 and 80°) in d_5-pyridine. At 60° the doublets began to collapse to single peaks and at 80° sharp singlets appeared at the midpoints of the previous doublets. A study of the Dreiding models of (+)-*N*-acetyl-*N*-demethyl-cyclomicrobuxeine shows that the rotation of *N*-methyl-*N*-acetyl group at C-3 is restricted by the C-4 exomethylenic hydrogens. Therefore the compound can exist as two conformers (**1a**) and (**1b**) at room temperature [7, 8]. The steric interaction appears to create a sufficiently large energy barrier between the two conformations so as to allow them to exist in two distinguishable states.

Extensive 2D-NMR experiments (COSY 45°, NOESY) [7] confirmed the above mentioned assignments. The overall spectral image thus resembled that for (+)-cyclomicrobuxeine [6] and in fact species **1** corresponds to the N_b-demethyl, N_b-acetyl derivative of (+)-cyclomicrobuxeine.

EXPERIMENTAL

Plant material. The leaves of *B. papillosa* (dry weight 50 kg) were collected from the northern regions of Pakistan in Sept. 1986.



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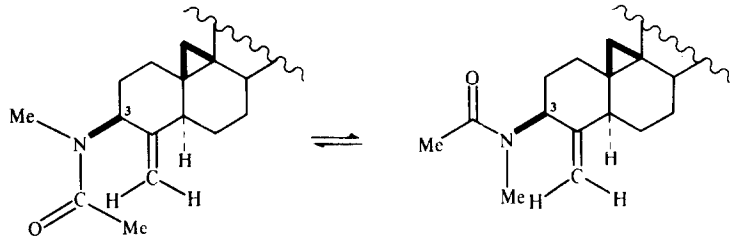

1a 1b

Extraction and purification. The EtOH extract of air-dried leaves was evapd to a gum. The total alkaloids were obtained by extraction into 10% AcOH. Partial separation of the alkaloids was achieved by extraction with $CHCl_3$ at different pH values. The fraction obtained at pH 3.5 (75 gm) was loaded on a silica gel column (3.0 kg). Elution was carried out with $CHCl_3$ and then with $CHCl_3$–MeOH. The main fraction thus obtained was further purified by prep. TLC (silica gel) in C_6H_{14}–$CHCl_3$–MeOH (10:10:1) to afford (+)-*N*-acetyl-*N*-demethylcyclomicrobuxeine (**1**) as an amorphous solid (5.5 mg), $[\alpha]_D^{20}$ +36° ($CHCl_3$); UV λ_{max}^{MeOH} nm 237 and 245; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1645 (amide and α,β-unsaturated carbonyl), 1595, (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ at room temp): δ0.11, 0.14 (1H, *d*, J = 4.2 Hz, H-19α), 0.38, 0.41 (1H, *d*, J = 4.2 Hz, H-19β), 0.99 (3H, *s*, Me), 1.19 (3H, *s*), 2.05, 2.14 (3H, *s*, NC(=O)Me), 2.26 (3H, *s*, C(=O)-Me), 2.88, 2.91 (3H, *s*, *N*-Me), 4.37, 4.58 (1H, *s*, =C-H), 4.43, 4.70 (1H, *s* =C-H), 6.65 (1H, *dd*, J_1 = 3.4 Hz, J_2 = 2.1 Hz, H-16); ^1H NMR (400 MHz, C_6H_5N-d_5 at 80°C): δ0.12 (1H, *d*, $J_{19\alpha,19\beta}$ = 4.3 Hz, H-19α), 0.45 (1H, *d*, $J_{19\beta,19\alpha}$ = 4.3 Hz, H-19β), 1.03 (3H, *s*, Me), 1.34 (3H, *s*, Me), 2.16 (3H, *s*, Me-21), 2.27 (3H, *s*, N-Me), 4.68 (1H, *s*, =C-H), 4.71 (1H, *s*, =C-H), 6.60 (1H, *dd*, J_1 = 3.4 Hz, J_2 = 2.2 Hz, H-16); MS *m/z* (rel *int.), 395.2910 ($C_{26}H_{37}NO_2$, calcd. 395.2824, 18), 380 $[M-Me]^+$ (12), 322 $[M-NH(Me)C(=O)\text{-}Me]^+$ (40), 74.0604 (C_3H_8NO, calcd 74.0605, 100), 57.0214 (C_2H_3NO, calcd 57.0214, 90).

REFERENCES

1. Atta-ur-Rahman, Choudhary, M. I., Ali, I. and Habib-ur-Rehman (1986), *J. Nat. Prod.* **49**, 106.
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, Alam, M., Choudhary, M. I. and Firdous, S. (1987) *Planta Med.* (in press).
6. Nakano, T., Terao, S. and Saeki, Y. (1966), *J. Chem. Soc.* (*C*), 1412.
7. Atta-ur-Rahman (1986) *Nuclear Magnetic Resonance Spectroscopy*, Springer, New York.
8. Atta-ur-Rahman, Ali, I. and Choudhary, M. I. (1985) *Z. Naturforsch.* **40b**, 543.