TAMYNINE - A NEW ALKALOID FROM *MURRAYA PANICULATA*

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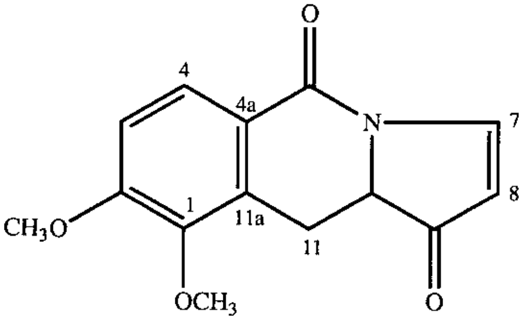
Abstract - A new alkaloid tamynine has been isolated from *Murraya paniculata.* Its structure has been assigned as 1 on the basis of chemical and spectral studies.

Murraya paniculata (Rutaceae), locally called "Orange Jasmine" or "China pox" is commonly found in Karachi, Pakistan as an ornamental plant. It is very popular for its green foliage and large clusters of fragrant flowers. It is propagated by seeds, cutting or layering. *Murraya paniculata* belongs to family Rutaceae which is spread out in the tropical and subtropical regions of South East Asia, Mediterranian countries, North America, Australia and South Africa. The leaves are useful for the treatment of diarrhoea, dysentry and posses antibiotic activity against the *Micrococcus pyogen* var. *aureus* and *E. coli.* Powdered leaves are applied to cuts. Twigs are used for dental cleaning purposes.[1-4] Earlier investigations on various parts of this plant led to the isolation of a few alkaloids, i.e. paniculidine, noracronycine, skimmianine and yuehchuchene.[5-7]

In view of our continued interest[8] in the chemical constituents of this plant, a reinvestigation in the leaves of this plant was undertaken. As a result of preliminary work on the leaves of *Murraya paniculata* a new crystalline alkaloid (Tamynine) has been isolated of which structure (**1**) has been assigned on the basis of extensive nmr studies.[9-12] The ^1H nmr assignments were made by means of ^1H-^1H, NOESY, COSY, J-resolved 2D homodecoupling and n.O.e experiments. The ^{13}C multiplicities were established by carrying out DEPT experiments.

RESULT AND DISCUSSION

The crude alkaloidal mixture obtained from the EtOH extract of *Murraya paniculata* was basified with ammonia and extracted with $CHCl_3$. The $CHCl_3$ extract was subjected to column chromatography. The



(1)

fraction obtained with hexane-acetone (9:1) was subjected to preparative tlc on silica gel GF_{254} plate to afford the alkaloid (**1**) as a crystalline material, mp 113-114°C, $[\alpha]_D^{26}$+ 103° (c = 0.23, $CHCl_3$). High resolution mass spectrum (HRms) of the alkaloid afforded molecular ion peak at m/z 259.08443 (calcd. as 259.08445) corresponding to the molecular formula $C_{14}H_{13}NO_4$, revealing 9 degree of unsaturation in the molecule. The molecular ion was confirmed by FAB mass spectrometry. Other prominent peaks in its mass spectrum were found to occur at m/z 229, 213, 189, 175, 131, 82, 69. The peak at m/z 229.0737 ($C_{13}H_{11}NO_3$) corresponded to the loss of methoxyl from the molecular ion. Its uv spectrum showed absorptions at 213 (log ε 4.419), 240 (4.378) and 305 nm (4.096), indicating a chromophore such as isoquinolone. The ir spectrum showed an intense absorptions at 1670 and 1710 cm^{-1} indicating the presence of an aromatic carbonyl and an enone.

The ^1H nmr spectrum ($CDCl_3$, 300 MHz) showed the presence of 13 protons in the molecule. A doublet at δ 6.86 (J = 7.8 Hz) was assigned to the C-3 proton. The C-4 proton appeared at δ 7.38 as a doublet (J = 7.8 Hz), its downfield chemical shift reflecting the presence of a carbonyl function at the peri position. The C-7 and C-8 olefinic protons appeared at δ 6.20 and 5.81 as doublets (J= 7.8 Hz). A multiplet at δ 4.33 was assigned to the C-10 proton, while the methylene protons at δ 3.90 and 3.97 were shown as double doublets. The C-11α and C-11β protons appeared as double doublets at δ 3.90 ($J_{11\alpha,11\beta}$ = 12.3 Hz, $J_{11\alpha,10\alpha}$ = 6.3 Hz), and δ 3.97 ($J_{11\beta,11\alpha}$ = 12.3, $J_{11\beta,10\alpha}$ = 6.3 Hz). The two methoxy groups appeared as singlets at δ 3.84 and 3.85. Two dimensional nmr measurements were carried out to verify the ^1H- nmr assignments. The coupling interactions were established through correlated spectroscopy (COSY) while the multiplicity of the overlaping proton signals was determined from the 2D J-resolved spectrum. The C-7 olefinic proton at δ 6.20 showed cross peaks with the C-8 proton at δ 5.81. The aromatic protons of C-3 and C-4 at δ 6.86 and 7.38 showed cross peaks each other. The NOESY spectrum served to establish the spatial proximities. The signal at δ 6.86 (C-3H) showed n.O.e interaction with that at δ 3.85 (2-OCH_3). *The signal at δ 3.90 (C-11αH) showed n.O.e interaction with* the C-11β proton at δ 3.97 in the NOESY spectrum. To record the subtle n.O.e effects which were not observed in the NOESY spectrum n.O.e difference measurements were carried out. Irradiation at δ 3.90 (C-11αH) resulted in 16.3% n.O.e between the signals at δ 3.84 (C-1 OCH_3) and 12.5% n.O.e at δ 3.97 (C-11βH). Irradiation at δ 3.85 (C-2 $0CH_3$) resulted in 8.2% n.O.e between the signals at δ 6.86 (C-3H) and 9.7% n.O.e at δ 3.84 (C-1 $0CH_3$), establishing the methoxy substitution at C-1 and again suggesting the presence of aromatic proton at C-4 δ 7.38. ^{13}C Nmr ($CDCl_3$, 75 MHz) spectrum showed presence of 14 carbon atoms in the molecule. The multiplicity assignments in the ^{13}C nmr spectrum were made by DEPT experiments, which revealed the presence of two methyl, one methylene and five methine carbons. The two methoxy carbons resonated at δ 56.4 and δ 56.1. The C-7 and C-8 olefinic carbons resonated at δ 113.9 and δ 107.8 while C-1 and C-2 appeared at δ 153.0 and 141.0. The C-10 resonated at δ 60.0 and C-11 at δ 23.3. The signal at δ 161.1 was assigned to the amidic carbonyl carbon and C-9 resonated at δ 160.0. The C-4 appeared at δ 127.2 its downfield chemical shift reflecting the deshielding influence of carbonyl function. The signals at δ 137.0 and 138.1 were assigned to the C-4a and C-11a. The C-3 resonated at δ 111.9.

EXPERIMENTAL

The plant material (20 kg) was collected from *suburbs* of the Karachi city and was identified by Prof. Dr. Mohammad Qaiser, Department of Botany, University of Karachi (voucher No. KUH 4378). The plant material was chopped into small pieces and extracted exhaustively with EtOH (40 l) for one week. The ethanolic extract was evaporated under reduced pressure. The material (65 g) thus obtained was washed with EtOAc. The aqueous layer was basified to pH-10 using 10% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure and subjected to column chromatography on SiO_2. The fraction obtained with n-hexane-Me_2CO (9:1) was subjected to preparative tlc on silica gel (GF-254) precoated plates with $CHCl_3$-MeOH (9.75:0.25) as a solvent system (Rf = 0.4). This afforded a pure crystalline alkaloid tamynine (1) mp 113-114°C, 14 mg, $[\alpha]_D^{26}$ + 103° (c = 0.23, $CHCl_3$; uv (MeOH) λ_{max} nm (log ε) 213 (4.419), 240 (4.378) and 305 (4.096); ir (KBr) 1670, 1710 cm^{-1}, ^1H nmr ($CDCl_3$, 300 MHz) δ 6.86 (1H, d, J= 7.8 Hz, C-3), 7.38 (1H, d, J= 7.8 Hz, C-4), 6.20 (1H, d, J= 7.8 Hz, C-7), 5.81 (1H, d, J= 7.8 Hz, C-8), 4.33 (1H, m, C-10), 3.90 (1H, dd, $J_{11\alpha,11\beta}$ = 12.3, $J_{11\alpha,10\alpha}$ = 6.3 Hz, C-11α), δ 3.97 (1H, dd, $J_{11\beta,11\alpha}$ = 12.3, $J_{11\beta,10\alpha}$ = 6.3 Hz, C-11β), 3.84 (3H, s, 1-OCH_3), 3.85 (3H, s, 2-OCH_3), ^{13}C nmr δ ($CDCl_3$, 75 MHz) 56.4 (1-OCH_3), 56.1 (2-OCH_3), 153.0 (C-1), 141.0 (C-2), 111.9 (C-3), 127.2 (C-4), 137.1 (C-4a), 161.1 (C-5), 113.9 (C-7), 107.8 (C-8), 160.0 (C-9), 60.0 (C-10), 23.3 (C-11), 138.1 (C-11a). Ir (KBr) ν_{max} cm^{-1} 1670 and 1710 (C=O), 1605 (C=C). HRms m/z M^+ 259.08445 $C_{14}H_{13}NO_4$. Elms m/z (rel. int.) $[M]^+$ 259 (100), $[M\text{-}OCH_2]^+$ 229 (48), $[M\text{-}(OCH_3+CH_3)]^+$, 213 (23), $[C_4H_4NO]^+$ 82 (11), 131 (5), 69 (13). FABms $[MH]^+$ 260 (100%).

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