# PLUMERININE - A NOVEL LUPIN ALKALOID FROM PLUMERIA RUBRA

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Abstract- Plumerinine, a novel lupin alkaloid has been isolated from the stem of Plumeria rubra and assigned structure 1 on the basis of spectral studies. This constitutes the first example of the occurrence of bicyclic lupin alkaloids in subfamily Plumerioideae of the Apocyanaceae.

Plumeria rubra (Apocyanaceae) commonly grows as ornamental plant in Southeast Asia particularly in Pakistan and India. It is reputed to possess purgative, diuretic, abortificient, antituberculotic properties, and is also used as remedy for rheumatism, diarrhoea, blennorrhea, gonorrhoea, syphilis, veneral sores, and leprosy.[1,2]

Enormous work[3-6] have been done on various parts of this plant, but no alkaloid has ever been isolated or characterised, although their presence has been reported in 1957 by Douglas and Kiang.[7]

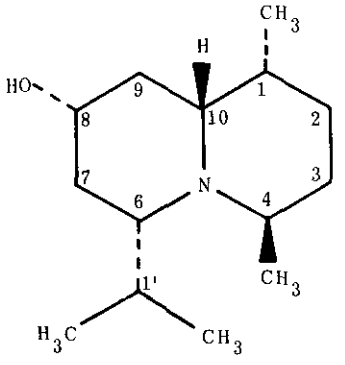
Herein we report the isolation and structural elucidation of a novel lupin alkaloid plumerinine from the freshly collected and shade dried stems. The structure 1 has been assigned to it on the basis of extensive spectral studies.

## RESULTS AND DISCUSSION

Plumerinine formed light brown viscous oil, showed $[\alpha]_D^{20}$ = 14.4° (c = 0.31, $CH_3OH$), and analysed for $C_{14}H_{27}NO$ (hrms, $M^+$ peak at m/z 225.2078; $C_{14}H_{27}NO$ requires 225.2094. It gave positive test with Dragendorff's reagent and the uv spectrum showed the presence of simple quinolizidine system[8] with $\lambda_{max}$ at 202 nm (log ε = 2.69) and a shoulder at 275 nm (log ε = 1.33). The ir spectrum showed a strong band at 3650 $cm^{-1}$ (OH group), a moderate band at 1150 $cm^{-1}$ (C-N stretch.), and another band at 1370 $cm^{-1}$ attributed to gem. dimethyl group. Bohlmann bands at 2820 and 2720 $cm^{-1}$ confirmed the presence of trans-fused quinolizidine system.[9] The broad band $^{13}C$-nmr spectrum showed 14 carbon atoms; their multiplicities determined through DEPT experiments keeping the last pulse angles θ = 45°, 90°, and 135°. It showed the presence of four methyl, four methylene, and six methine carbons. The molecular formula of plumerinine corresponded to two degree of unsaturation, and the absence of unsaturation in ir, $^1H$- and $^{13}C$-nmr revealed bicyclic lupin type skeleton. The fragmentation pattern studied with the help of link-scan measurements was in complete agreement to lupin alkaloids.[10] The presence of alcoholic function and an isopropyl

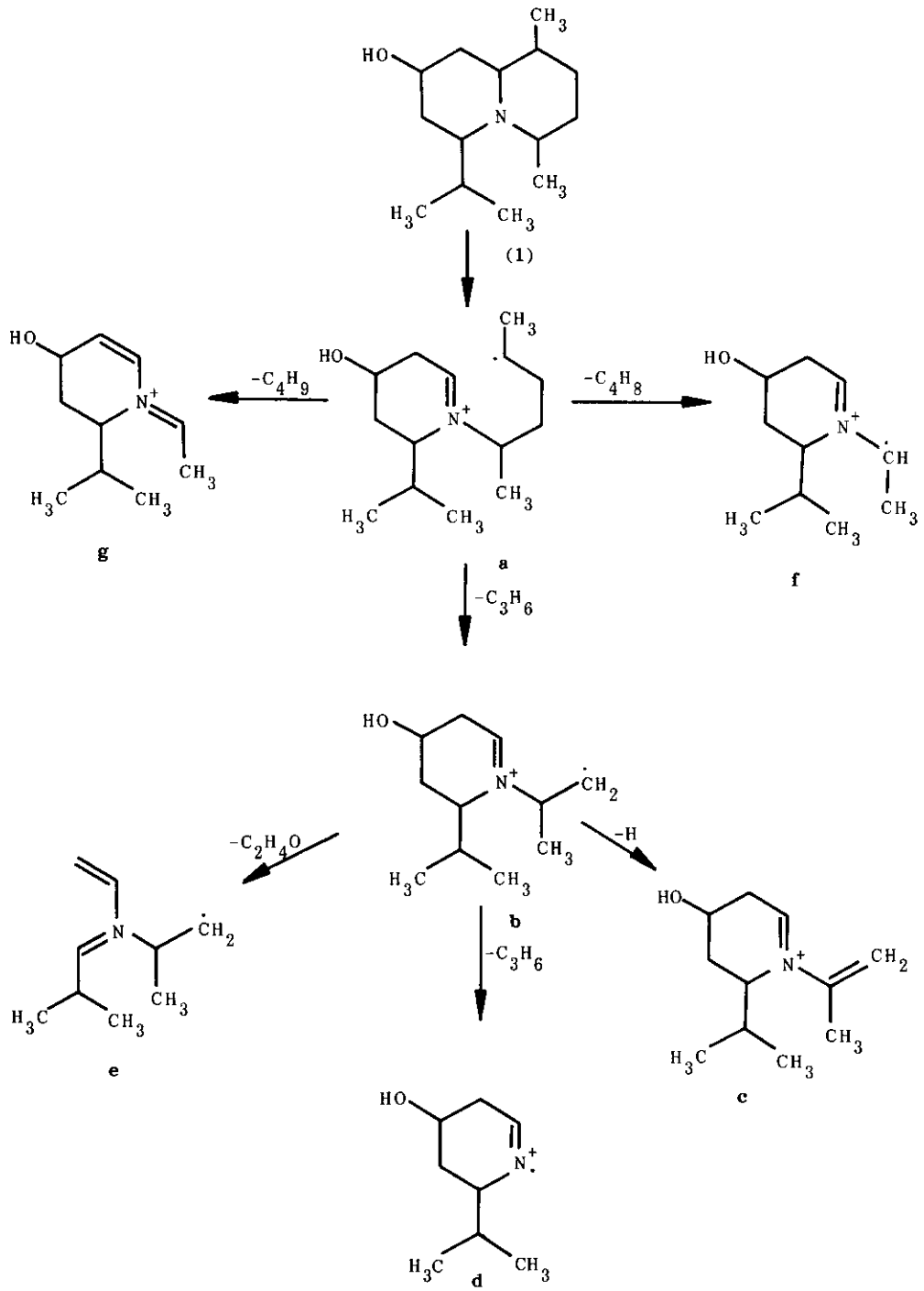
moiety was indicated by the loss of $H_2O$ (m/z 207.1979, $C_{14}H_{25}N$) as well as isopropyl (m/z 182.1544, $C_{11}H_{20}NO$) from the parent ion. The α-cleavage in the ring yielded ion 'a' which is decomposed further by loss of $C_3H_6$ unit to give a fragment 'b' at m/z 183.1618 ($C_{11}H_{21}NO$). This fragment further eliminated a hydrogen atom to form ion 'c' at m/z 182.1544 ($C_{11}H_{20}NO$) or propylene to form ion 'd' at m/z 141.1152 ($C_8H_{15}NO$). Another fragment 'e' arose from "b" by retro-Diels-Alder process at m/z 139.1361 ($C_9H_{17}N$). The loss of isopropyl group from ion 'e' gave another fragment at m/z 96.0860 ($C_6H_{10}N$). In quinolizidines where one of the ring is unsubstituted, the ions "b", and 'd' are observed at m/z 111 and 83, respectively[10], which indicated that in plumerinine one of the rings contained both the alcoholic and isopropyl functionalities, the former at C-7 or C-8 and the latter at C-6 or C-9. Alternative bond rupture of 'a' with or without loss of an additional H-atom has been invoked to explain the genesis of fragments of masses 168.1388 ($C_{10}H_{18}NO$) and 169.1469 ($C_{10}H_{19}NO$). The quinolizidines in which one of the ring is unsubstituted and the carbon adjacent to N in the other ring is also unsubstituted, the corresponding ions are observed at m/z 96 and 97, respectively.[10] Since the hydroxyl and isopropyl moieties have already been accounted in one of the rings, the C-4 must therefore carry a methyl group. The facile cleavage of bond between C-1 and C-10 allowed us to place the remaining methyl group at C-1. On the basis of further evidences described below, the positions 6 and 8 have been assigned to isopropyl and hydroxyl groups; the fragmentation pattern of plumerinine could thus be represented by Scheme 1. The structure and stereochemistry at chiral centres were confirmed on the basis of $^1$H- and $^{13}$C-nmr, homodecoupling experiments, $^1$H-$^1$H homonuclear correlated spectroscopy (COSY-45°) and $^1$H-$^{13}$C heteronuclear correlated spectroscopy (Heterocosy). The most downfield signal in the $^{13}$C-nmr spectrum at δ 72.95 was assigned to the carbon carrying the hydroxyl group. It showed a cross peak in heterocosy at δ 3.20. The 1-H signal at δ 3.20 was found to be a multiplet in $^1$H-nmr spectrum in which axial-axial coupling of 10.84 Hz and axial-equatorial coupling of 3.00 Hz was clearly visible. This allowed us to assign axial orientation to the carbinylic proton. Another downfield signal at δ 72.82 could be assigned to C-10. The normal shift of this carbon in unsubstituted quinolizidines ranges from 63-66[11] ppm and the downward shifting here could be attributed to the presence of methyl group on the adjacent carbon. The alkyl induced paramagnetic shifts of this magnitude have already been reported in literature.[12,13] This signal showed a cross peak in heterocosy with signal at δ 3.12 which was therefore assigned to H-10. Irradiation at δ 3.12 simplified the 2H-multiplet at δ 1.91 and also 1-H multiplet at δ 2.85. On the other hand, irradiation at δ 2.85 caused the multiplet at δ 3.12 to collapse into a dd ($J_{aa}$ = 10.72Hz, $J_{ae}$ = 3.04Hz), also simplified the 2H-multiplet at δ 1.78, and collapsed the methyl doublet at δ 1.12 into a singlet. The signals at δ 1.91, δ 2.85, and δ 1.78 could, therefore, be assigned to $H_2$-9, H-1, and $H_2$-2, respectively. Irradiation of methyl group at δ 1.12 simplified the multiplet at δ 2.85 in which

no axial-axial coupling could be observed, confirming the β- and equatorial orientation of H-1. Since irradiation at H-1 caused the multiplet of H-10 to collapse into a double doublet showing axial-axial and axial-equatorial coupling, the configuration at H-10 must be β- and axial. Irradiation of $H_2$-9 at δ 1.91 again collapsed the multiplet of H-10 into a doublet ($J_{ae}$ = 3.17 Hz). Consequently the isopropyl group must be at C-6 and not C-9. Both the methyls of isopropyl group formed a doublet at δ1.13 which in turn showed a cross-peak with 1H multiplet at δ 2.91. The latter in turn showed a cross peak with 1H multiplet at δ 3.40. Irradiation at δ 2.91 collapsed the 6H doublet at δ 1.13 into a singlet and simplified the multiplet at δ 3.40 into a double doublet ($J_{aa}$ = 11.27 Hz, $J_{ae}$=2.91 Hz). On the other hand, irradiation at δ 3.40 simplified the multiplets at δ 2.91 and δ 1.82, respectively. These experiments not only helped in assigning signals of δ 3.40 and δ 1.82 to H-6 and $H_2$-7, but also confirmed the absence of hydroxyl group at C-7 and the orientation of isopropyl group at C-6 as α- and equatorial. The equatorial hydroxylic group must therefore be at C-8 in α-orientation. The position of hydroxyl group was authenticated by irradiation of the carbinylic proton at δ 3.20 which simplified the multiplets of $H_2$-7 and $H_2$-9 at δ 1.82 and δ 1.91, respectively, into double doublets. The remaining downfield multiplet at δ 3.30 could be assigned to H-4. Its irradiation caused the doublet of methyl group at δ 1.26 to collapse into a singlet and also simplified the 2H multiplet of $H_2$-3. The irradiation at δ 1.26 caused the multiplet at δ 3.30 to collapse into a double doublet ($J_{ae}$ = 2.73 Hz, $J_{ee}$ = 2.13Hz). The proton at C-4 could therefore be assigned α- and equatorial-orientation. The biogenesis of plumerinine can be explained in the light of various pathways already described in literature for lupin alkaloids.[14] It arises either from two molecules of suitably derivatized lysine or from 2-isopropyl-4-hydroxy-piperidine and a suitable $C_6$-dicarbonyl compound. To the best of our knowledge this is the first report of the natural occurrence of bicyclic lupin alkaloids in the subfamily Plumerioideae of the Apocyanaceae and its isolation is of chemotaxonomic significance.




(1)

**Scheme 1**



## EXPERIMENTAL

Uv and ir spectra were recorded on Shimadzu UV-240 and JASCO A-302 spectrophotometers respectively. Hrms were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) Computer system. Nmr spectra were recorded on a Bruker AM-300 spectrometer with TMS as internal reference. Tlc experiments were performed on silica gel (PF-254, 0.2 mm) plates (E.Merck). Two dimensional COSY-45° experiment was acquired at 300 MHz with sweep width of 4000 Hz (2 k data points in $\omega_2$) and 2000 Hz (256$t_1$ values zero-filled to 1k) in $\omega_1$. The heteronuclear two dimensional $^1H$-$^{13}C$ chemical shift correlation experiments were carried out at 300 MHz with sweep width of 12820 Hz (2k data points in $\omega_2$) and 1024 Hz (256$t_1$ values zero-filled to 1k) in $\omega_1$. In both the 2D-experiments a 2 sec. relaxation delay was used, 16 transients were performed for each $t_1$ value.

Isolation of Plumerinine (1): The fresh plant material (stem, 20 kg) was collected from the Karachi region and was identified by a Plant Taxonomist, Department of Botany, University of Karachi. The stems were chopped into small pieces and extracted thrice with MeOH (60 l). The combined methanolic extract was evaporated under reduced pressure and the resulting residue (225 g) was acidified with 10% acetic acid (1 l) and then washed with $CHCl_3$. The acidic aqueous layer was basified with 20% $NH_4OH$ and exhaustively extracted with $CHCl_3$. The alkaloidal residue (8 g), recovered from the $CHCl_3$ layer, was chromatographed over silica gel (300 g) and successively eluted with increasing polarities of mixture of hexane, chloroform, and methanol. The hexane:chloroform (1:1) and chloroform eluants gave two and three spots on tlc, respectively, but could not be worked-up further due to paucity of material and extreme susceptibility to aerial oxidation. Plumerinine was eluted with chloroform:methanol (9:1) and was purified by preparative tlc with chloroform:methanol:ammonia (7:3:0.5) as solvent system. The major alkaloid, plumerinine (1) (Rf 0.3) (85 mg) was separated as a viscous oil.

Uv: ($CH_3OH$) $\lambda_{max}$ 202, 275 nm. Ir: (KBr) $\nu_{max}$ 3650, 3350 $cm^{-1}$ (OH), 1150 $cm^{-1}$ (C-N stretch.), 1370 $cm^{-1}$ (isopropyl), 2820 and 2720 $cm^{-1}$ (Bohlman bands). Hrms: $M^+$ 225.2078 ($C_{14}H_{27}NO$), 207.1979 ($C_{14}H_{25}N$), 183.1618 ($C_{11}H_{21}NO$), 182.1544 ($C_{11}H_{20}NO$), 168.1388 ($C_{10}H_{18}NO$), 141.1152 ($C_8H_{15}NO$), 139.1361 ($C_9H_{17}N$), 96.0860 ($C_6H_{10}N$).

$^1H$-Nmr ($CD_3OD$, 300 MHz): $\delta$ 3.40 (1H, m, H-6), 3.30 (1H, m, H-4), 3,20 (1H, m, H-8), 3.12 (1H, m, H-10), 2.91 (1H, m, H-1' of isopropyl), 2.85 (1H, m, H-1), 2.58 (1H,br s,OH), 1.78 (2H,m,$H_2$-2), 1.26 (3H, d, J = 7.20Hz, $CH_3$-C4), 1.12 (3H, d, J = 6.38Hz, $CH_3$-C1), 1.13 (6H, d, J = 6.44Hz, $2CH_3$, isopropyl). $^{13}C$-Nmr ($CD_3OD$, 75 MHz): $\delta$ 72.95 (C-8), 72.82 (C-10), 68.58 (C-6), 67.08 (C-4), 49.58 (C-1), 49.46 (C-1', isopropyl), 41.32 (C-9), 41.11 (C-7), 41.04 (C-3), 37.97 (C-2), 18.30 ($CH_3$-C4), 21.93 ($CH_3$-C1), 21.98 ($2CH_3$-isopropyl).

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