ALKALOIDS FROM THE LEAVES OF ALSTONIA MACROPHYLLA

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Abstract - Alstonia macrophylla Wall, of Sri Lankan origin has yielded a new picraline alkaloid, "alstopicralamine" (1), the structure of which was established by using modern spectroscopic techniques. A known base vincamajine (2) was also isolated for the first time from the leaves of A.macrophylla.

Alstonia macrophylla Wall, family Apocynaceae, grows extensively in Sri Lanka and in southern India. Previous chemical investigations have resulted in the isolation of several monomeric or dimeric indole bases[1-10]. Present studies describe the isolation of a new picraline indole base alstopicralamine (1), along with a known base vincamajine (2). The latter has been previously found in Vinca major and other Vinca sp., Alstonia spectabilis, A.libanotica, A.quaternata, Cabucala torulosa etc.[11-13].

The crude alkaloidal compounds were isolated from the weakly basic alkaloidal fractions of A.macrophylla leaves extracted between pH 8-9.

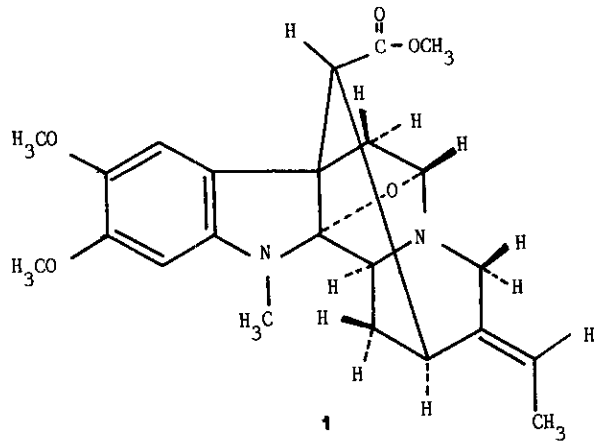
Alstopicralamine (1) has the molecular formula $C_{23}H_{28}N_2O_5$ as established by high resolution ms indicating eleven degrees of unsaturation in the molecule. Its uv spectrum showed absorptions at 230, 245, 300 and 307 nm[14] revealing the presence of a dihydroindole (indoline) system. The ir spectrum displayed intense absorptions at 1723 (ester carbonyl), 1600 (C=C) and 1280 (C-O) cm^{-1}.

The ^{1}H-nmr spectrum of the compound (400 MHz, $CDCl_3$) bore a distinct similarity with those of picraline[15] and picralinal[16] indicating that it possessed a closely related structure. It showed

three-proton doublet of doublets at δ 1.51 ($J_{18,19}$= 7.0 Hz, $J_{18,21}$= 2.4 Hz) assigned to the 18-vinylic methyl group, while the vicinal C-19 vinylic proton appeared as a quartet at δ 5.52 ($J_{19,18}$= 7.0 Hz). A three-proton singlet at δ 2.94 was due to the N-CH_3 protons. The spectrum interestingly showed three three-proton singlets at δ 3.65, 3.75 and 3.86 corresponding to two methoxy groups and one carbomethoxy group. The absence of coupling in the two one-proton signals in the aromatic region at δ 6.31 and 6.74 established their para disposition, thereby indicating the ortho substitution pattern of the remaining two methoxy groups on the aromatic nucleus of the indoline moiety, at C-9 and C-10. An interesting close doublet at δ 5.01 (J = 2.6 Hz) was also observed, its chemical shift being characteristic for a hydrogen attached to a carbon bearing a lactone or ester oxygen. By comparing the chemical shift of this proton with the corresponding proton in picralinal[16] and picraline[15], it was possible to assign it to C-5H. The low coupling constant of the signal indicated that coupling was observed only with C-6αH. A broad singlet at δ 3.36 was ascribed to the C-3α methine proton by comparison of its chemical shift with the corresponding proton in picraline (H3α, δ 3.30). A doublet at δ 2.40 ($J_{16,15}$= 3.5 Hz) was due to the C-16 methine proton, while the vicinal C-15 methine proton appeared at δ3.35 as a multiplet. The C-21α methylenic proton appeared as doublet at δ3.30 ($J_{21\alpha,21\beta}$= 17.4 Hz) while the C-21β proton resonated as a doublet of doublets at δ3.90 ($J_{21\beta,21\alpha}$= 17.4 Hz, $J_{21\beta,18}$ = 2.4 Hz) showing geminal coupling with C-21αH and homo-allylic coupling with the 18-CH_3 protons of the ethylidene side chain. The C-6β methylenic proton appeared as a doublet of doublets at δ2.29 ($J_{6\alpha,6\beta}$= 14.3 Hz, $J_{6\alpha,5}$= 2.6 Hz) while a doublet at δ 3.40 ($J_{6\beta,6\alpha}$= 14.3 Hz) was due to the C-6α proton. The C-14α methylenic proton appeared at δ1.90 as multiplet while the geminal C-14β proton resonated at δ 2.18 as a multiplet. The chemical shift of the carbomethoxy singlet (δ3.65) indicated that it bears 'R' configuration being pointed away from the shielding influence of the aromatic nucleus. The 2D COSY 45° spectrum[17] confirmed above mentioned connectivities as indicated in fig. 1.

The mass spectrum of compound 1 displayed the molecular ion at m/z 412.1813 as the base peak, in agreement with the formula $C_{23}H_{28}N_2O_5$ (calcd. 412.1998). A peak at m/z 397.1742 represented the loss of methyl group from the molecular ion. Another peak at m/z 353.1834 arose by the loss of carbomethoxy group from M^+. A large (42%) peak at m/z 135.1156 (calcd. for $C_9H_{13}N$, 135.1047) corresponded to the substituted piperidine ion, while the ion at m/z 276 represented the loss of this fragment from the molecular ion. Other peaks were observed at m/z 313, 197, 136, 122, 121, 108 and 83. The overall fragmentation pattern were distinctly similar to those of other picralima bases[18]. The above studies led to structure 1 for this new picralima base. Like other picralima bases,

it may also arise in nature from 5α-carboxystrictosidine. An additional ring is formed by an ether linkage across ring C. This could occur by hydration of the immonium group, followed by attack of the hydroxy group on the indolenine double bond[18].



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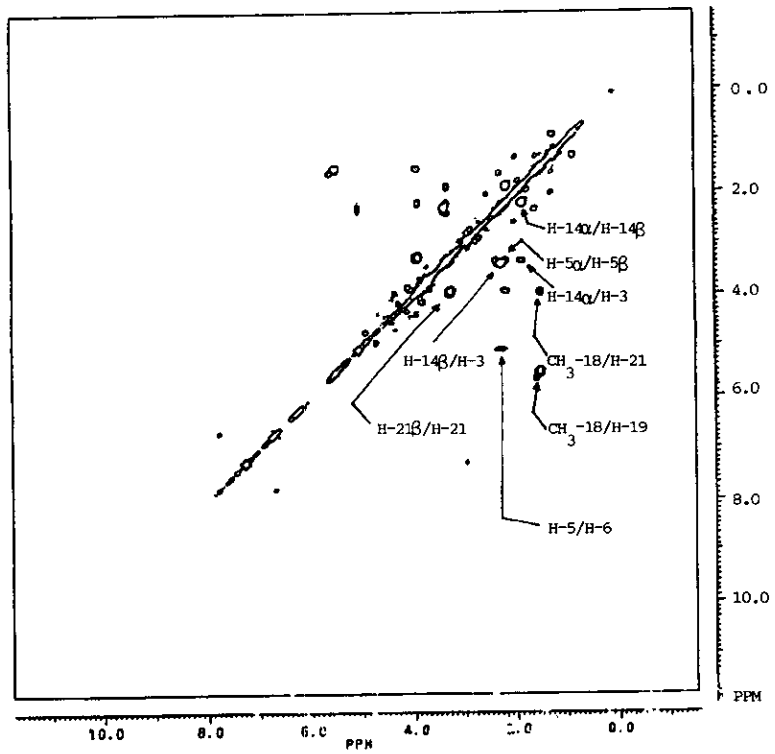
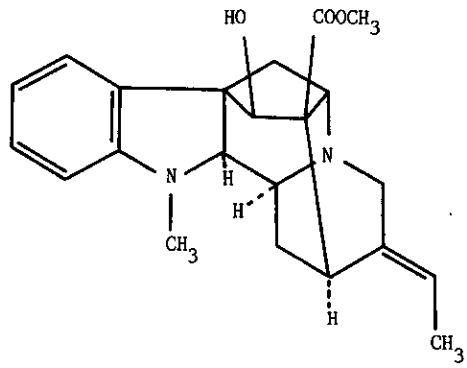


Fig. 1: COSY 45° spectrum of (+)-alstopicralamine (1)

Our second compound was identified as vincamajine (2) by comparison of its spectral data (uv, ir, ^{1}H-nmr, ms) with that reported in the literature.[11-13] The compound was isolated first from Vinca major and later from other Vinca sp. Alstonia spectabilis, A. libanotica, A.quaternata, Cabucala torulosa etc.[11-13] but has been isolated for the first time from A. macrophylla.



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EXPERIMENTAL

Plant Material - The leaves of A.macrophylla (dry weight 30 kg) were collected from Colombo district, Sri Lanka, in Nov. 1985. The plant was identified by Prof. S. Balasubramanium, Department of Botany, University of Paradeniya, Sri Lanka.

Extraction and Purification - Extraction was carried out with EtOH at room temperature. The solvent was evaporated in vacuo to afford a gum (30 g) which was taken up in 10% HCl. The pH was then adjusted by addition of 20% NH_4OH. The fraction obtained by extraction with $CHCl_3$ at pH 9.0 was evaporated and subjected to column chromatography on silica gel. Elution was with increasing polarities of $CHCl_3$-MeOH. An important fraction was obtained by eluting with $CHCl_3$-MeOH (90:10) which was subjected to tlc (silica gel) in solvent system C_6H_{14}:$(CH_3)_2CO$ (6:4) to afford compounds **1** and **2.**

Alstopicralamine (**1**)-$[\alpha]_D^{20}$ = + 3.33° (c = 0.016 g/100 ml in $CHCl_3$); λ_{max}^{MeOH} nm 230, 245, 300, 307 ; $\nu_{max}^{CHCl_3}$ cm^{-1} 1723, 1600, 1280 cm^{-1} ; ^{1}H-nmr (400 MHz, $CDCl_3$) δ 1.51 (3H, dd, $J_{18,19}$= 7.0 Hz, $J_{18,21\beta}$= 2.4 Hz, CH_3-18), 1.90 (1H, m, H-14α), 2.18 (1H, m, H-14β), 2.29 (1H, dd, $J_{6\alpha,6\beta}$= 14.3 Hz, $J_{6\alpha,5}$ = 2.6 Hz, H-6α), 2.40 (1H, d, $J_{16,15}$= 3.5 Hz, H-16), 2.94 (3H, s, N-CH_3), 3.30 (1H, d, $J_{21\alpha,21\beta}$= 17.4 Hz, H-21α), 3.35 (1H, m, H-15), 3.36 (1H, brs, H-3), 3.40 (1H, $J_{6\beta,6\alpha}$= 14.3 Hz, H-6β), 3.65 (3H, s, C-OCH_3), 3.75 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 5.01 (1H, d, $J_{5,6}$= 2.6 Hz, H-5),

3.90 (1H, dd, $J_{21\beta,21\alpha}$= 17.4 Hz, $J_{21\beta,18}$= 2.4 Hz, H-21β), 5.52 (1H, d, $J_{19,18}$= 7.0 Hz, H-19), 6.31 (1H, s, H-11), 6.74 (1H, s, H-8); ms m/z (rel. int.) : 412.1813 ($C_{23}H_{28}N_2O_5$, calcd. 412.1998, 100), 397.1742 ($C_{22}H_{25}N_2O_5$, calcd. 397.1763), 353.1834 ($C_{21}H_{25}N_2O_3$, calcd. 353.1865), 313 (90), 276 (45), 197 (40), 136 (32), 135.1156 ($C_9H_{13}N$, calcd. 135.1047, 42), 122 (20), 121 (36), 108 (45).

Vincamajine (2) - $[\alpha]_D$ = -48° (c = 0.078 g/100 ml in $CHCl_3$) ; λ_{max}^{MeOH}nm 245, 292 ; $\nu_{max}^{CHCl_3}$cm^{-1} 1725 (ester carbonyl), 1608 (C=C), 1295 (C-O) ; ^{1}H-nmr (400 MHz, $CDCl_3$) δ 1.62 (3H, dd, $J_{18,19}$= 7.0 Hz, $H_{18,21}$= 1.3 Hz, CH_3-18), 1.50 (1H, dd, $J_{3,14}$ = 9.8 Hz, $J_{14\alpha,14\beta}$= 14.4 Hz, H-14α), 2.50 (1H, dd, $J_{14\beta,15}$ = 4.4 Hz, $J_{14\beta,14\alpha}$= 14.4 Hz, H-14β), 2.64 (3H, s, N-CH_3), 2.66 (1H, dd, $J_{5,6}$= 4.8 Hz, $J_{6\alpha,6\beta}$= 11.0 Hz, H-6α), 3.30 (1H, d, $J_{2,3}$ = 5.0 Hz, H-2), 3.52 (2H, m, H-21), 3.67 (3H, s, C(=O)-OCH_3), 4.25 (1H, brs, OH), 5.67 (1H, q, J = 7.0 Hz, H-19), 6.65 (1H, d, $J_{12,11}$= 7.9 Hz, H-12), 6.78 (1H, ddd, $J_{11,12}$= 7.9 Hz, $H_{11,10}$= 7.8 Hz, $J_{11,9}$= 1.0 Hz, H-11), 7.12 (1H, dd, $J_{9,10}$= 7.2 Hz, $J_{9,11}$= 1.0 Hz, H-9), 7.20 (1H, ddd, $J_{10,11}$= 7.8 Hz, $J_{10,9}$= 7.2 Hz, $J_{10,12}$= 0.9 Hz, H-10) ; ms m/z (rel. int.) 366.1918 ($C_{22}H_{26}N_2O_3$, calcd. 366.1943 ,60), 334 (10), 291 (10), 263 (8), 222 (40), 190 (60), 157 (100), 148 (65), 131 (15), 115 (12), 97 (20), 83 (28).

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Received, 30th November, 1987