THE ISOLATION AND STRUCTURE OF ERVATICINE, A NEW INDOLE ALKALOID FROM *ERVATAMIA CORONARIA*

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Abstract - A new 2-acylindole alkaloid "ervaticine" was isolated from the leaves of Ervatamia coronaria and its structure was determined as (I) on the basis of spectral studies.

Ervatamia coronaria Stapf (Apocynaceae) is a glabrous, evergreen tree found abundantly in the gardens of West Pakistan. The plant is used in the indigenous system of medicine for the treatment of opthalmia, for application on wounds and inflammed parts of the body, as anthelmintic, etc. Anticancer activity has been shown by the crude extracts of the plant[1]. A number of indole alkaloids have previously been reported by us from its leaves[2-6].

The crude alkaloids (20 gm) obtained from the ethanolic extracts of the fresh leaves (50 kg) were subjected to pH fractionation. The fraction obtained at pH 1.0 afforded a number of alkaloids which were further purified by column chromatography and preparative t.l.c. to afford the new alkaloid, ervaticine, as a light yellow amorphous material (8 mg), $[\alpha]_D$= + 120° ($CHCl_3$)

The compound afforded a U.V. spectrum characteristic of 2-acylindoles, showing absorption *maxima at 235 nm* (log ε 4.15) *and 312 nm* (log ε 4.20) *and minimum* at 265 nm (log ε 3.05). The IR spectrum (KBr) afforded peaks at 3400 cm^{-1} (N-H), 2900 cm^{-1} (C-H), 1640 cm^{-1} (C=O) and 1580 cm^{-1} (C=C).

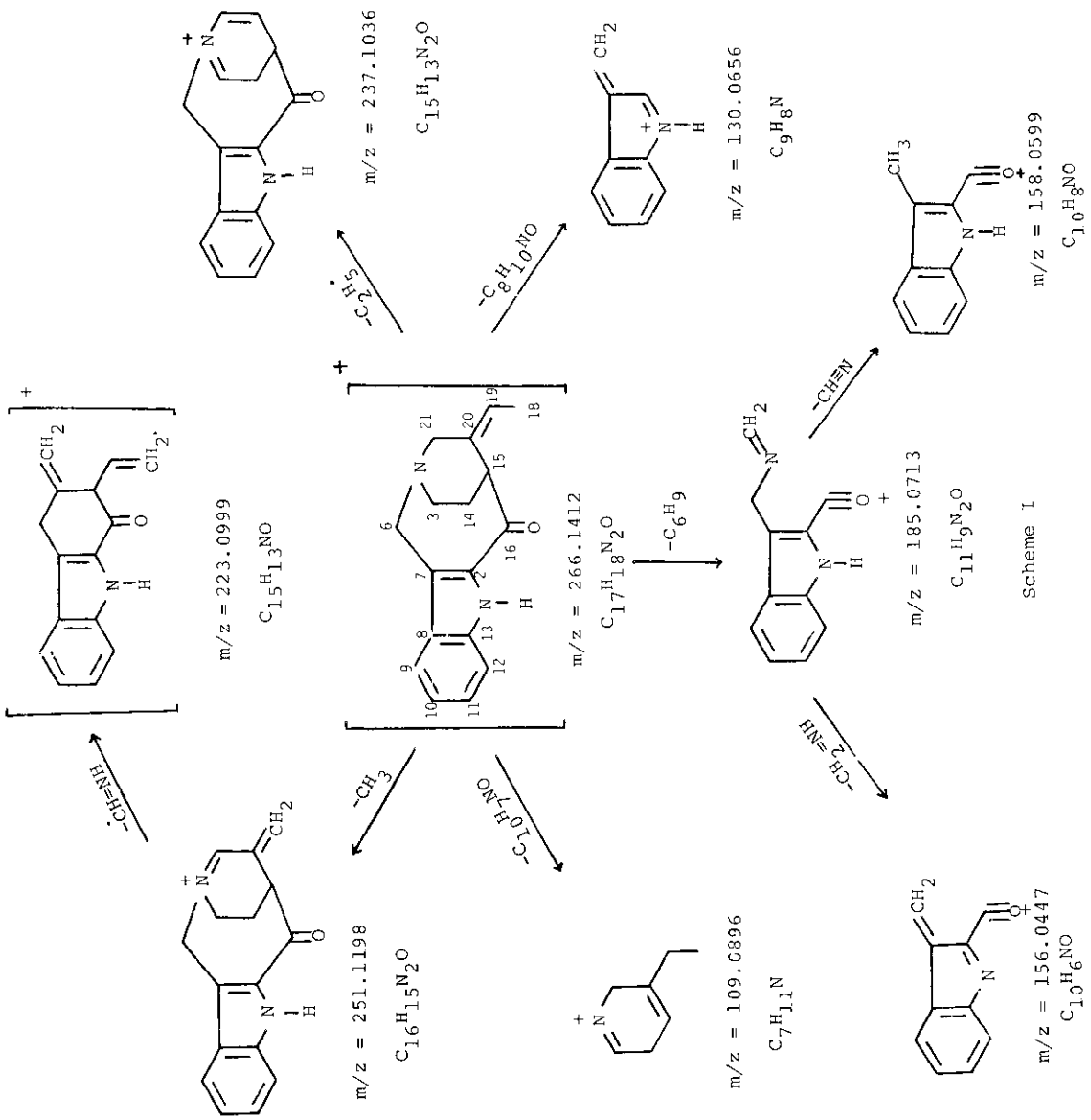
The mass spectrum of ervaticine showed the molecular ion at m/z = 266.1412 which was consistent with the molecular formula $C_{17}H_{18}N_2O$ indicating the presence of ten double bond equivalents in the molecule. Seven of these were accounted for by the presence of the 2-acylindole system. The mass fragmentation pattern of ervaticine was very similar to that of vallesamine[7]. The following major peaks were observed in its mass spectrum: 266.1412 (M^+, $C_{17}H_{18}N_2O$, 51.2%), 251.1198 (M^+-CH_3, $C_{16}H_{15}N_2O$, 13.3%), 237.1036 (M^+-C_2H_5, $C_{15}H_{13}N_2O$, 60.7%), 223.0999 ($C_{15}H_{13}NO$, 22.9%), 185.0713 ($C_{11}H_9N_2O$, 25.8%), 158.0599 ($C_{10}H_8NO$, 19.5%), 156.0447 ($C_{10}H_6NO$, 41.3%) 130.0656 (C_9H_8N, 100%) and 109.0896 ($C_7H_{11}N$, 77.3%). The formulae of the ions were established by computer monitored accurate mass measurements and confirmed by peak matching experiments on important ions. Linked scan measurements of metastable transitions resulting from the fragmentation of the molecular ion at m/z 266 showed that it fragmented directly to the ions at m/z 251, 237, 223, 185, 130 and 109. The ion at m/z 251 was shown to give rise to the ion at m/z 223. The ions at m/z 156 and 158 were seen to arise directly from the ion at m/z 185. These fragmentations are presented in Scheme I.

The ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of ervaticine was strikingly similar to that of vallesamine[8]. Two sets of AB double doublets showing large geminal couplings were observed at δ 4.28, δ4.79 (J = 18.6 Hz) and δ 3.33, δ 3.87 (J = 15.9 Hz), which were assigned to the C-6α, C-6β and C-21α, C-21β protons respectively. The C-19 olefinic proton appeared as a quartet at δ 5.49 ($J_{19,18}$=6.9 Hz). One three-proton doublet at δ 1.52 ($J_{18,19}$=6.9Hz) was assigned to the C-18 methyl protons. The C-15 proton appeared as a doublet at δ 3.98 ($J_{15,14}$=6Hz) while the C-14α and C-14β protons appeared as multiplets at δ 2.08 and δ2.30 respectively. A multiplet at δ 3.10 was assigned to the C-3α proton while the C-3β proton appeared as another multiplet at δ3.40. The N-H proton resonated as a broad singlet at δ 8.92. Examination of the aromatic region of ervaticine showed that the C-9 proton appeared as a doublet at δ 7.57 ($J_{9,10}$= 7.5 Hz) while the C-10 proton appeared as a doublet of double doublets at δ7.11 ($J_{10,9}$= 7.5 Hz, $J_{10,11}$=5.4 Hz and $J_{10,12}$=2.4Hz). Another doublet of double doublets at δ7.10 was assigned to the C-11 proton ($J_{11,10}$=5.4 Hz, $J_{11,12}$=5.1 Hz and $J_{11,9}$=2.4 Hz). The C-12 proton appeared as a doublet at δ 7.33 ($J_{11,12}$=5.1 Hz). The assignments of all the protons and their couplings were confirmed by carrying out a series of homodecoupling experiments at all chemical shifts determined by the projection of the 2D-J resolved spectrum. Irradiation of the olefinic proton at δ 5.49 resulted in the collapse of the methyl doublet into a singlet. Similarly the quartet at δ 5.49 collapsed into a singlet on irradiation of the methyl protons at δ 1.52. Irradiation of the C-14 α proton resulted in the collapse of the doublet at δ 3.98 into a singlet as well as in a simplification of the multiplets at δ 2.3 and δ 3.1 due to C-14β and C-3α protons respectively. The doublet at δ 4.79 collapsed into a singlet on irradiation of the C-6α proton at δ 4.28. Irradiation of the doublet at δ 3.33 resulted in the collapse of the doublet due to the C-21β proton at δ 3.87 into a sinlget. The inter-relationships were confirmed by 2-D-NMR (COSY-45°) experiments, which showed prominent cross peaks at the expected positions.

The ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz) of ervaticine provided strong support for the proposed structure (I). The methyl carbon appeared as a low field signal at δ 12.75. The signal at δ 44.2 was assigned to the C-15 carbon which is α to the carbonyl group. The C-14 carbon resonated at δ 29.7. Another signal at δ48.0 was assigned to the C-3 methylene carbon atom. The two low field methylenes resonating at δ 53.2 and δ 54.9 were assigned as C-6 and C-21 carbons. The signal at δ126.7 was assigned to the C-19 olefinic carbon atom. The low field signals at δ 120.3, 120.9, δ 126.6 and δ111.6 were assigned to the C-9, C-10, C-11 and C-12 carbons respectively. The signals of the quaternary carbon atoms were too weak to be recorded.

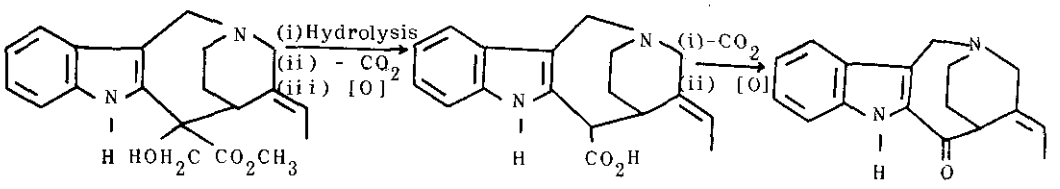
Irradiation of the methyl protons of the ethylidene group resulted in 6.57% nOe at the C-21β proton at δ 3.87. Irradiation of the olefinic proton at C-19 (δ5.49) resulted in a corresponding 12% nOe at the C-15 proton. These results served to establish 'Z' stereochemical disposition of the ethylidene group.

Reduction of ervaticine with sodium borohydride in methanol resulted in the formation of dihydro-ervaticine, a slower moving compound which gave a normal indolic u.v. spectrum. In view of the above data, structure (I) is assigned to ervaticine.


-CH=NH
m/z = 223.0999
$C_{15}H_{13}NO$
-CH_3
-$C_{10}H_7NO$
m/z = 251.1198
$C_{16}H_{15}N_2O$
m/z = 109.0896
$C_7H_{11}N$
-C_2H_5
m/z = 237.1036
$C_{15}H_{13}N_2O$
-$C_8H_{10}NO$
m/z = 266.1412
$C_{17}H_{18}N_2O$
-C_6H_9
m/z = 130.0656
C_9H_8N
-CH_2=NH
-CH=N
m/z = 185.0713
$C_{11}H_9N_2O$
m/z = 158.0599
$C_{10}H_8NO$
m/z = 156.0447
$C_{10}H_6NO$

Scheme I

Ervaticine is the first 2-acylindole alkaloid with one of the aliphatic carbon atoms of the ethylamine side chain of the tryptophan precursor missing. It probably arises by the same biogenetic path in the plant as vallesamine[9], (III), which then undergoes hydrolysis, decarboxylation and oxidation to give the acid (II) which can then undergo decarboxylation and oxidation to afford ervaticine (I) as shown in Scheme II.



(III) (II) (I)

Scheme II

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