ISOLATION OF 19,20-Z-VALLESAMINE AND 19,20-E-VALLESAMINE FROM ALSTONIA SCHOLARIS

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Abstract - A new alkaloid 19,20-Z-Vallesamine along with 19,20-E-vallesamine has been isolated from the leaves of Alstonia scholaris and the stereochemistry has been deduced from NOE measurements.

Alstonia scholaris is a large ornamental tree widely distributed in Pakistan. Various indole alkaloids has been previously isolated from this plant.[1-8] Investigations into the alkaloidal constituents in the leaves of this plant have led to the isolation of a new alkaloid isomeric to vellasmine which possesses the rare Z configuration at the 19,20 double bond. Besides this 19,20-E-vallesamine[9] was also isolated for the first time from this plant.

RESULTS AND DISCUSSION

The new alkaloid (1) was isolated from the crude alkaloidal mixture obtained from the alcoholic extract of the leaves of A. scholaris. It was obtained as a colourless amorphous solid, $[\alpha]_D$ + 182° ($CHCl_3$). The UV spectrum was found to be characteristic for the indole chromophore, showing absorption maxima at λ_{max} 225, 275, 282 and 293 nm. The IR spectrum showed absorptions at ν_{max} 3300 cm^{-1} (NH) and 1725 cm^{-1} (ester C=O). It was found to have the molecular ion at m/z 340.1947 corresponding to the formula $C_{20}H_{24}N_2O_3$ indicating 10 double bond equivalents in the molecule. Other significant peaks were observed at m/z 208, 143 and 122. The mass spectral fragmentation pattern was identical to that reported for vallesamine[9].

The ^{1}H-NMR (300 MHz) in $CDCl_3$ showed one doublet at δ 1.69 ($J_{18,19}$ = 6.4Hz) for the ethylidene methyl group. An AB double doublet at δ 4.93 (d, $J_{6\alpha,6\beta}$=16.4 Hz) and δ 4.05 (d, $J_{6\beta,6\alpha}$ = 16.4Hz) were assigned to H-6α and H-6β protons respectively. The H-15 proton appeared as a multiplet centered at δ 3.60 while H-21α and H-21β protons resonated together at δ 3.60 as multiplets. The H-14α and H-14β protons appeared as multiplets at δ 2.14 and δ 2.01 respectively. Another set of AB doublets resonated at δ 4.20 and δ 3.80 ($J_{17\alpha,17\beta}$ = 10.2 Hz)

Table-1

^{1}H-NMR data for 19,20-Z-vallesamine and 19,20-E-vallesamine ($CDCl_3$)

		19,20-Z-vallesamine		19,20-E-vallesamine	
NH	bs	10.22		9.50	
H-3α H-3β	m	2.95-2.85		2.96-2.85	
H-6α	d	4.93	$J_{6\alpha 6\beta}$=16.44Hz	4.82	$J_{6\alpha,6\beta}$=17.16Hz
H-6β	d	4.05	$J_{6\beta,6\alpha}$= 16.44Hz	4.09	$J_{6\beta,6\alpha}$= 17.13Hz
H-9	bd	7.44	$J_{9,10}$ =7.8Hz	7.17	$J_{9,10}$ = 6.9Hz
H-10	t	7.12	$J_{10,11}$= $J_{10,9}$ =7.08Hz	7.07	$J_{10,11}$=$J_{10,9}$=7.00Hz
H-11	t	7.03	$J_{11,10}$,$J_{11,12}$=7.6Hz	7.3	$J_{12,11}$ = 7.9
H-12	bd	7.27	$J_{12,11}$ =10.59Hz	7.3	$J_{12,11}$=7.9Hz
H-14 α	m	2.19		2.33	
H-14 β	m	2.01		1.89	
H-15	m	3.66		3.63	
H-17α	d	4.20	$J_{17\alpha,17\beta}$= 10.20Hz	4.19	$J_{17\alpha,17\beta}$= 10.86Hz
H-17β	d	3.80	$J_{17\beta,17\alpha}$= 10.20Hz	3.81	$J_{17\beta,17\alpha}$= 10.83Hz
H-18	d	1.69	$J_{18,19}$ = 6.4Hz	1.74	$J_{18,19}$ = 6.93Hz
H-19	q	5.52	$J_{19,18}$=6.3Hz	5.56	$J_{19,18}$=6.6Hz
H-21α H-21β	m	3.60		3.60	
$COOCH_3$	s	3.70			

which were assigned to H-17α and H-17β protons respectively. The ester methyl group appeared as a singlet at δ3.70 while the olefinic proton resonated at δ 5.52 as a quartet ($J_{19,18}$ = 6.3 Hz) (Table-1). The coupling interactions were determined through COSY 45° spectrum[10] (fig. 1) while the multiplicities of the protons signals were unambiguously determined from the 2D-J resolved spectrum[11]. On the basis of this data, the gross structure (1) could be assigned to the alkaloid.

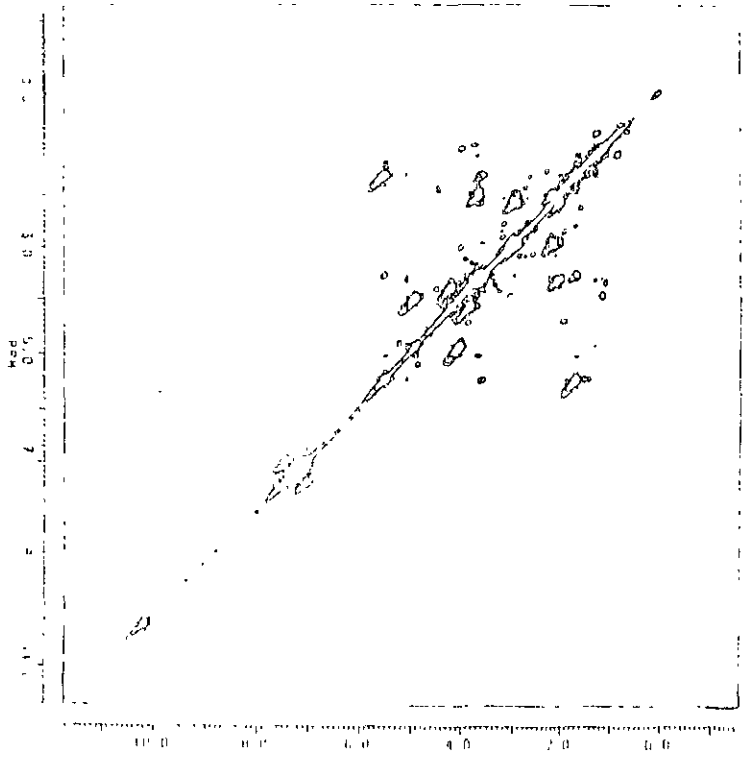


Fig. 1 COSY 45° spectrum of 19,20-Z-Vallesamine

The ^{13}C-NMR spectrum (75 MHz in $CDCl_3$) of the alkaloid showed 20 carbons resonances. The multiplicity of each carbon atom was determined by using DEPT experiments with the polarization pulse θ = 45°, 90°, and 135°. The experiments revealed the presence of one methyl carbon, five methylene carbons and six methine carbons, in agreement with strucuture (1). The chemical shifts of (1) were similar to those reported in the literature[12] for "vallesamine". The major difference appeared at the C-19 and C-20 carbons which were shifted by 3.32 ppm downfield and 4.62 ppm

upfield respectively which indicated a change in the stereochemistry at the 19,20 double bond (Table II).

Table-II

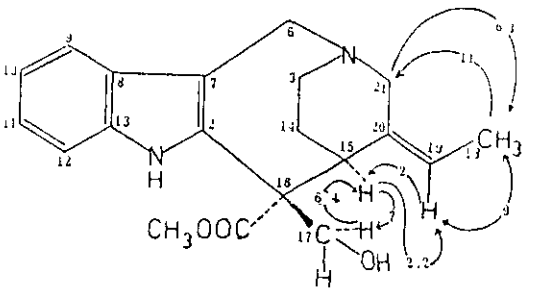
^{13}C-NMR chemical shifts for 19,20-Z-vallesamine and 19,20-E-vallesamine ($CDCl_3$)

19,20-Z-vallesamine				19,20-E-vallesamine			
Carbon No.	Chemical Shifts	Carbon No.	Chemical Shifts	Carbon No.	Chemical Shifts	Carobon No.	Chemical Shifts
2	134.36	13	135.06	2	133.62	13	137.47
3	47.28	14	22.22	3	47.47	14	23.81
6	49.15	15	35.40	6	51.21	15	36.27
7	109.29	16	58.80	7	109.15	16	48.54
8	128.21	17	70.00	8	128.17	17	70.17
9	117.90	18	14.17	9	118.35	18	14.04
10	119.55	19	127.42	10.	119.13	19	124.01
11	122.56	20	127.78	11	122.36	20	132.40
12	111.04	21	52.36	12	110.65	21	54.04
		$\underline{C}OOCH_3$	174.11			$\underline{CO}OCH_3$	175.20
		$COOCH_{\underline{3}}$	52.94			$COOCH_{\underline{3}}$	52.87

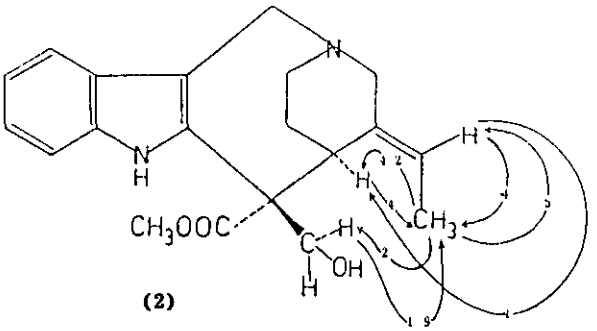
In order to confirm the relative stereochemistry at various centres NOE difference measurements were carried out. Irradiation of the H-18 doublet at δ1.69 gave 9.0 % NOE of the signal at δ5.52 for the H-19 proton 11% NOE of the multiplet at δ 3.60 for the H-21α and H-21β protons. This established the proximity of H-18, H-19 and H-21α and H-21β protons. On the other hand, irradiation on H-21α and H-21β protons at δ3.60 resulted in 6.3% NOE at the H-18 proton. These results showed that the 19,20 double bond has Z configuration. An NOE of 6.4% for H-15 was observed upon irradiation of the proton at δ 4.20 (H-17 α). The observed NOE'S are given in fig.2. On the basis of this data the alkaloid was assigned the structure corresponding to 19,20-Z-vallesamine (1).

"Vallesamine" (2) was also isolated for the first time from this plant. The alkaloid was identified by spectral data (UV, I.R., ^{1}H-NMR and ^{13}C-NMR)[12]. The previous proposal for the structure of vallesamine was based on its chemical studies[9], but the stereochemistry of the ethylidene group had not been established by spectroscopic methods. In order to confirm the relative stereochemistry at various centres, NOE difference experiments were carried out. Irradiation of the H-18 doublet at δ1.74 gave a 4% increase in the area of the multiplet at δ3.65 assigned to the H-15 proton as well as 2% increase of the doublet at δ 4.20 and 5% increase of the quartet at δ 5.52 which were

assigned to H-17α and H-19 respectively. No NOE was found between H-18 and H-21 protons. Irradiation of the H-17 quartet resulted in 2% NOE of the H-18 protons at δ 1.77 as well as 4% NOE of the H-15 proton at δ 3.76. These measurements serve to establish that vallesamine bears "E" stereochemistry at the 19,20 double bond.



(1)



(2)

Fig. 2

EXPERIMENTAL

Optical rotations were measured in $CHCl_3$ on Polartronic Universal Australian standard K 157 digital polarimeter, UV spectra were recorded on a Shimadzu UV 240 spectrometer, IR spectra were recorded on Jasco A-302 spectrometer. Mass spectra were recorded on MAT 312 mass spectrometer connected to DEC PDP 11/34 computer. ^{1}H-NMR spectra were recorded at 300 MHz and ^{13}C-NMR at 75MHz in $CDCl_3$ with TMS as internal standard on Bruker AM-300 PFT NMR spectrometer. DEPT experiments were carried out with polarisation pulse θ=45°, 90° and 135°. For NOE difference studies, the sample solution was degassed by freeze-thaw cycles on a good high vacuum line and the normal spectrum was substracted from the enhanced spectrum so that only differences were recorded.

Plant Material

The plant material was collected in Karachi and identified by Prof. S.I. Ali, Department of Botany, University of Karachi, Pakistan.

Extraction and Isolation

The powedered leaves (80 kg) were extracted with MeOH (100 litre). The methanolic extract was concentrated by evaporation under reduced pressure at 40°C to yield 3 kg of a crude concentrate, which was dissolved in 5% HCl and shaken with pet.ether and ethyl acetate in order to remove fatty acids and non alkaloidal material. The aqueous acidic layer was basified with ammonia and extracted with chloroform at different pH values.

Isolation of 19, 20-Z-Vallesamine

The fraction obtained at pH 9 (0.5 g) was subjected to column chromatography over aluminium oxide (90 mesh, 1 kg). Elution was carried out with increasing polarities of pet.ether-acetone. The fraction obtained on elution with pet. ether-acetone (7:3) (100 mg) was subjected to prep.TLC using pet.ether-acetone-ammonia (1:1:0.1) to afford an alkaloid 19,20-Z-vallesamine (1), mp 210°C, [α] + 182° ($CHCl_3$), UV (MeOH) λ_{max} 225, 275, 282 and 293 nm; IR ($CHCl_3$)ν_{max} 3300 cm^{-1} (NH) and 1725 cm^{-1} (ester C=O), MS m/z 340.1947 (calcd. for $C_{20}H_{24}N_2O_3$), 339 (10) 310 (40) 309 (28) 208 (5), 194 (7); 143 (10), 122 (80) ^{1}H-NMR see table I, ^{13}C-NMR see table II.

Isolation of 19, 20-E-Vallesamine

The fraction obtained at pH-4 (0.3 kg) was subjected to column chromatography over silica (F254, mesh 60). Elution was carried out with increasing polarities of pet.ether-acetone. The fraction obtained on elution with pet.ether-acetone (1:1) (150 mg) was subjected to prep.TLC using pet.ether acetone-ammonia (1:1:0.01) to afford an alkaloid 19,20-E-vallesamine, mp 162°C, [α] + 165 ($CHCl_3$), UV (MeOH) λ_{max} 223, 284 and 292 nm, IR ($CHCl_3$) ν_{max} 3400, 2820 and 1720 cm^{-1}, MS (rel.int.) 340.1772 ($C_{20}H_{24}N_2O_3$) 339 (11), 310 (35), 309 (17), 208 (10), 194 (14), 143 (13), 122 (79); ^{1}H-NMR (see table I) ^{13}C-NMR (see table II).

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