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INDOLE ALKALOIDS FROM *ALSTONIA SCHOLARIS*

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Key Word Index—*Alstania scholaris*; Apocynaceae; leaves; indole alkaloids; rhazimanine; alstonamine.

Abstract—A new indole alkaloid, alstonamine and a sitsirikine type indole alkaloid, rhazimanine, have been isolated from the leaves of *Alstonia scholaris*.

INTRODUCTION

As a part of our continuing programme on the isolation and structural studies on chemical constituents of medicinal plants, we wish to report here the isolation and structure of a new alkaloid alstonamine (**1**) from the leaves of *Alstonia scholaris* along with the known alkaloid rhazimanine (**2**) [1], which has not been previously reported from this plant.

RESULTS AND DISCUSSION

The crude alkaloidal mixture obtained from the alcoholic extract of the leaves of *A. scholaris* was selectively extracted with chloroform at different pH values. The fraction obtained at pH 9 was subjected to CC followed by prep. TLC. This resulted in the isolation of two alkaloids: a slower moving new alkaloid named alstonamine (**1**), and the faster moving rhazimanine (**2**) [1] which has not been previously reported from this plant.

Alkaloid (**1**) was obtained as a colourless amorphous solid $[\alpha]_D^{20} + 46$ ($CHCl_3$, *c* 2). The UV spectrum was characteristic of the indole chromophore showing maxima at 222, 283, 290 and minima at 249, 287 nm. The IR spectrum showed absorptions at 3300 cm^{-1} (NH) and 1725 cm^{-1} (ester C=O). The EI-mass spectrum showed the $[M]^+$ at *m/z* 338 which was confirmed by FD-mass spectrometry. The empirical formula was established by high resolution mass spectrometry as $C_{20}H_{22}N_2O_3$ (measured 338.1632, calc. 338.1630). Other significant peaks were observed at *m/z* 307, 251, 206, 157, 170 and 122. From the ^{1}H NMR and mass spectral data it was apparent that alstonamine (**1**) was closely related to vallesamine [2]. The $[M]^+$ of (**1**), however, was 2 mu less than that of vallesamine [2] suggesting the presence of an extra degree of unsaturation.

The ^{1}H NMR spectrum (300 MHz) of alstonamine (**1**) was very similar to that reported for vallesamine [3]. The main difference was the absence of the signals for the methyl protons and the presence of two broadened doublets centred at δ4.21 (18βH, $J_{18\beta,18\alpha}$ = 14.25 Hz, $J_{18\beta,19}$ = 3.4 Hz) and δ4.50 (18αH, $J_{18\alpha,18\beta}$ = 14.2, $J_{18\alpha,19}$ = 5.42 Hz) which were assigned to the 18β and 18α protons, respectively. The C-6α and C-6β protons were found to resonate as sets of AB doublets at δ4.87 and δ4.02, respectively, ($J_{6\alpha,6\beta}$ = 16.29 Hz). Another set of AB doublets occurred at δ4.38 and δ3.85 which were assigned to the H-17α and H-17β protons, respectively ($J_{17\alpha,17\beta}$ = 12.45 Hz). The ester methyl resonated as a 3 H singlet at δ3.88 while the olefinic protons resonated as a multiplet at δ5.53. The close correspondence of these ^{1}H NMR signals with those of vallesamine, the absence of the 18-methyl protons and the presence of an additional double doublet (broadened by homoallylic coupling) for the C-18 methylene protons indicated that the C-18 carbon had undergone cyclization with the C-17 hydroxyl group to generate a new 7-membered ring in alstonamine.

In order to confirm the assignments in the ^{1}H NMR spectrum a comprehensive series of homodecoupling experiments was carried out. Irradiation of the doublet for the H-18β proton at δ4.21 led to a collapse of the H-18α proton to a simple doublet ($J_{18\alpha,19}$ = 3.42 Hz) with the disappearence of the larger geminal coupling of 14.25 Hz. It also led to a simplification of the multiplet at δ3.45 for the C-21α/C-21β protons and of the multiplet at δ3.69 of the H-15 proton. Irradiation at the chemical shift for the 17α proton (δ4.38) resulted in the collapse of the doublet at δ3.85 due to the H-17β proton into a singlet. The chemical shifts, coupling constants and proton–proton connectivities were confirmed by recording a 2D-COSY 45 spectrum [4] (Fig. 1) and 2D-*J* resolved spectrum [5].

Both alstonamine (**1**) and vallesamine displayed similar chemical shifts for the aromatic carbons in the ^{13}C NMR spectrum (Table 2). The methylene carbons C-17 and C-18 adjacent to the ethereal oxygen appeared at δ70.99 and δ77.15, respectively. The carbons C-6, C-3 and C-21 α to N_b showed resonances at δ49.40, 43.32 and 55.43, respectively. The ester methyl occurred at δ52.45, while the

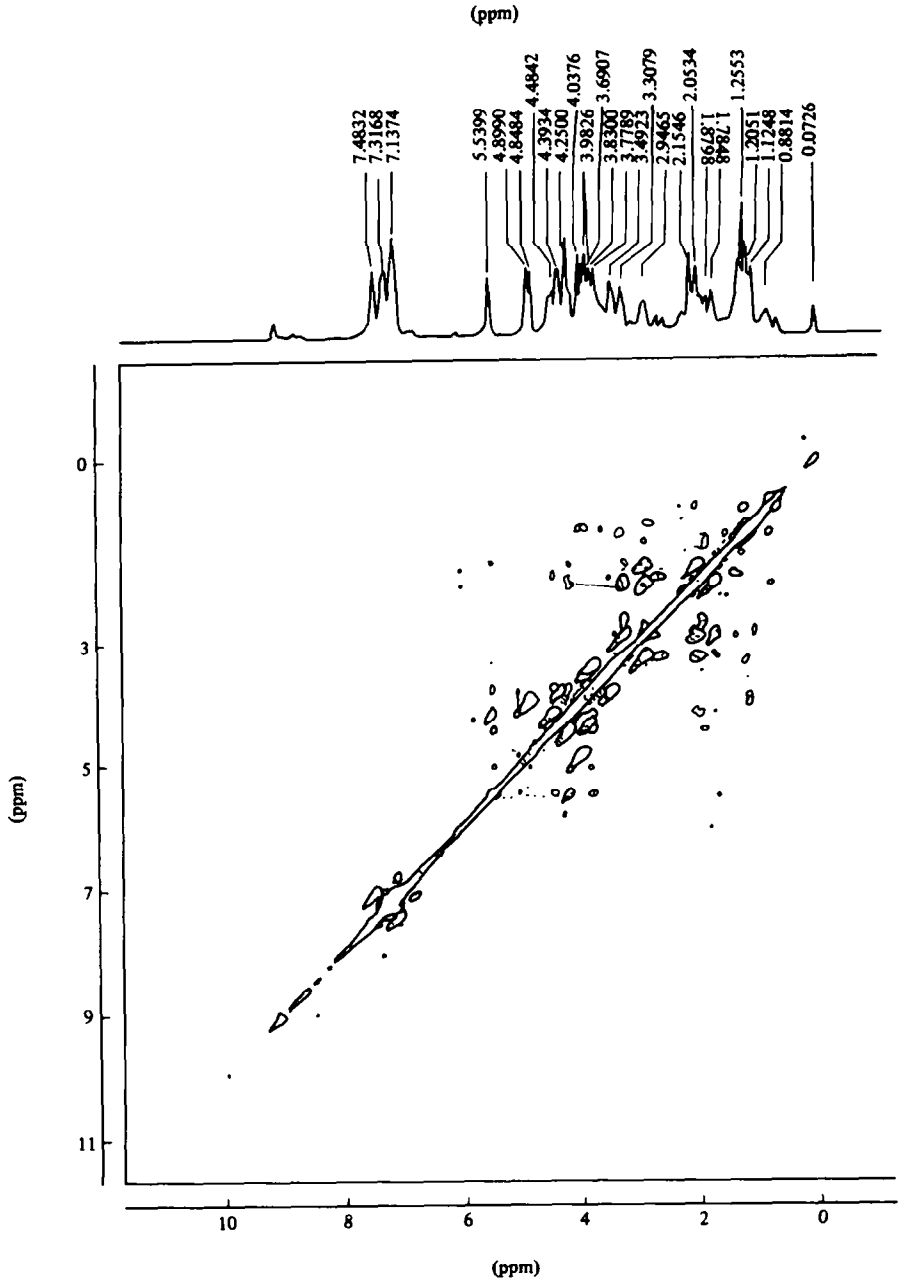
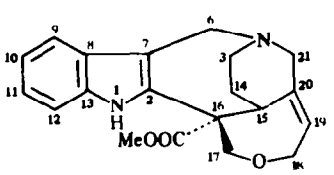


Fig. 1. 2D-COSY 45 spectrum of alstonamine (1).



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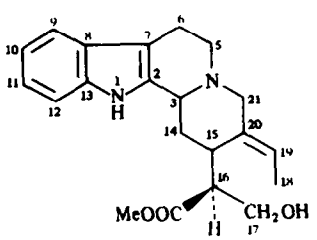

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Table 1. ^{1}H NMR data of alstonamine (1)

Protons	Chemical shift	Integration	Coupling constant (Hz)
NH	9.25 *s*	1 H	
H-3α H-3β	2.93 *m*	2 H	
H-6β	4.02 *d*	1 H	$J_{6\beta,6\alpha} = 16.29$
H-6α	4.87 *d*	1 H	$J_{6\alpha,6\beta} = 16.2$
H-9	7.48 *d*	1 H	$J_{9,10} = 7.8$
H-10	7.17 *ddd*	1 H	$J_{10,9} = 7.8$, $J_{10,11} = 7.4$ $J_{10,12} = 1.2$
H-11	7.08 *ddd*	1 H	$J_{11,12} = J_{11,10} = 7.68$ $J_{11,9} = 1.2$
H-12	7.31 *d*	1 H	$J_{12,11} = 7.92$
H-14α	1.76 *dd*	1 H	$J_{14\alpha,14\beta} = 15.22$, $J_{14\alpha,3} = 8.25$
H-14β	2:14 *m*	1 H	
H-15	3.69 *m*	1 H	
H-17α	4.38 *d*	1 H	$J_{17\alpha,17\beta} = 12.45$
H-17β	3.85 *d*	1 H	$J_{17\beta,17\alpha} = 12.45$
H-18β	4.21 *dd*	1 H	$J_{18\beta,18\alpha} = 14.25$, $J_{18\beta,19} = 3.42$
H-18α	4.50 *dd*	1 H	$J_{18\alpha,18\beta} = 14.25$, $J_{18\alpha,19} = 3.42$
H-19	5.53 *bm*		
H-21α H-21β	3.45 *m*		

Table 2. ^{13}C NMR of alstonamine (1) and vallesamine

Carbon no.	(1)	Multiplicity (DEPT)	Vallesamine	Multiplicity (DEPT)
2	132.00	–C–	133.11	–C–
3	43.32	CH_2	47.40	CH_2
6	49.44	CH_2	50.40	CH_2
7	106.34	–C–	107.7	–C–
8	128.66	–C–	128.0	–C–
9	118.20	CH	118.10	CH
10	119.99	CH–	119.00	CH
11	122.75	CH	122.2	CH
12	110.91	CH	110.60	CH
13	135.10	–C–	135.0	–C–
14	20.55	CH_2	23.7	CH_2
15	35.78	CH	36.0	CH
16	58.15	–C–	58.8	–C–
17	71.15	–C–	70.0	CH_2
18	77.15	CH_2	14.00	CH_3
19	124.59	CH	124.80	CH
20	132.11	–C–	132.5	–C–
21	55.40	CH_2	53.5	–C–
22	55.40	CH_2	53.5	CH_2
$COOCH_3$	52.45	CH_3	53.80	
$COOCH_3$	174.98		175.20	

carbonyl group resonated at δ174.98. The multiplicity assignments were made by employing DEPT pulse sequence with the last polarization pulse angle being kept at $\theta = 135$, 90 and 45°.

Alstonamine (1) probably arises from the C-18 hydroxylated precursor through an intramolecular cyclization reaction with the C-17 hydroxyl function.

Another alkaloid isolated from *A. scholaris* was rhazimanine (2) which has not been previously isolated from this plant. The alkaloid was identified by spectral data (UV, IR, ^{1}H NMR and ^{13}C NMR and mass spectrometry) [1] and co-TLC with an authentic sample.

EXPERIMENTAL

General. ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR at 75 MHz in $CDCl_3$ with TMS as int. std; DEPT expts were carried out with the polarization pulse $\theta = 45°, 90°$ and 135°. Optical rotations were measured in $CHCl_3$.

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

Extraction and isolation. Powdered leaves (80 kg) were extracted with MeOH (75 l.). The alcoholic extract was concd by evapn under red. pres. at 40° to yield 10 kg of a crude concentrate which was dissolved in 5% HCl (10 l.) and shaken with petrol and EtOAc in order to remove fatty and non-alkaloidal components. The aq. acidic layer was extracted with $CHCl_3$ at different pH values. The fraction obtained at pH 9 (5 kg) was subjected to CC over Al_2O_3 (90 mesh, 1 kg). Elution was carried out with mixtures of petrol–Me_2CO of increasing polarity. The fraction obtained on elution with petrol–Me_2CO (4:1) (100 mg) was subjected to TLC using petrol–Me_2CO–NH_3 (1:1:0.01) to afford two alkaloids alstonamine (1) (30 mg) and rhazimanine (50 mg). Alstonamine (1), mp 126°, $[\alpha]_D^{27}$ 46° (*c* 2 in $CDCl_3$); UV λ_{max} 222, 283, 290 λ_{min} 249, 287 nm; IR 3300 cm^{-1} (NH) 1725 cm^{-1} (ester C=O). MS *m/z* (rel. int.) 338.1632 $[M]^+$, (60) ($C_{20}H_{22}N_2O_3$, cal. 338.1630), 307 (10), 279 (40), 251 (60), 206 (70), 170 (30), 157 (47), 149 (19), 68 (100). ^{1}H NMR (see Table 1). ^{13}C NMR (see Table 2).

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