NMR Studies on Alioline – A Novel Vinca Alkaloid

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Extensive NMR studies on the novel vinca alkaloid alioline (**1**) have been carried out. These studies include 1D NMR experiments, *i.e.* ^{1}H-^{1}H decoupling, NOED, ^{13}C NMR (broad-band and DEPT) as well as 2D homonuclear (COSY, NOESY and *J*-resolved) and 2D-hetero COSY experiments.

Key words: Iboga Alkaloid, Alioline, NMR Studies

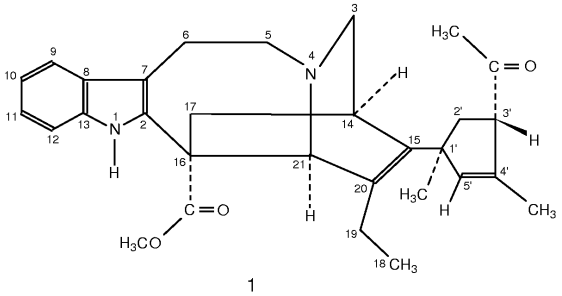
Introduction

Extensive NMR studies on the vinca alkaloid alioline (**1**) led us to assign unambiguously the chemical shifts of all protons and carbons and also the relative stereochemistry of each of the asymmetric centres of the molecule. These studies include 1D NMR experiments as well as 2D homonuclear and heteronuclear NMR experiments [1 – 6]. The ^{1}H NMR assignments were made with the help of COSY-45 and 2D heteronuclear correlated spectroscopy experiments while the ^{13}C multiplicities were established by DEPT pulse sequences and confirmed by 2D hetero-COSY experiments [6 – 8]. The 2D NOESY experiments and a series of NOE difference measurements [7, 8] were used to determine the stereochemistry of all asymmetric centres in the molecule. The NMR shift assignments were also compared to the reported data of similar compounds [9 – 18].

Results and Discussion

A novel vinca alkaloid, alioline (**1**), was isolated from *Catharanthus roseus* [19]. Its UV spectrum was typical of the indolic chromophore while the IR spectrum showed the presence of indolic N-H, aliphatic C-H, ester C=O, ketonic C=O, C=C and aromatic C-H functionalities in the molecule. The high resolution mass spectrum showed the molecular ion at $m/z = 472.2720$, consistent with the molecular formula $C_{30}H_{36}N_2O_3$, indicating fourteen double bond equivalents in the molecule.

The ^{1}H NMR spectrum ($CDCI_3$, 300 MHz) indicated the presence of 36 protons each of which



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was identified with the help of extensive two-dimensional NMR experiments (COSY-45, 2D *J*-resolved, NOESY), NOE difference measurements and correlated with the corresponding carbon atoms through the 2D-heteronuclear shift correlated (Hetero-COSY) spectra [2 – 5]. The ^{1}H NMR spectrum of alioline (**1**) showed a three-proton triplet at $\delta = 0.27$ ($J_{18,19\alpha} = J_{18,19\beta} = 7.4$ Hz). Its rather upfield chemical shift is attributed to it falling in the shielding zone of the olefinic bond in the five-membered ring. The 19α and β protons appeared at $\delta = 1.17$ and 1.32, respectively, as a double doublet showing geminal coupling ($J_{19\alpha,19\beta} = J_{19\beta,19\alpha} = 13.6$ Hz) and vicinal coupling ($J_{19\beta,18} = 7.4$ Hz) with the 18-Me protons. The coupling showed the non-equivalence of the methylene protons of the ethyl side chain [20, 21]. A broad singlet at $\delta = 2.58$ was assigned to the 21-H, its upfield chemical shift suggesting α-stereochemistry [20, 21]. A three-proton singlet at $\delta = 3.78$ was assigned to the ester methyl protons, while two other singlets at $\delta = 1.45$ and 1.80 were assigned to the 4′-methyl and the acetyl methyl protons on the five-membered ring, respectively. The 1′-methyl protons appeared at $\delta = 0.66$ as a singlet,

Table 1. ^{1}H NMR shift assignments for alioline (**1**).

Proton No.	Chemical shift (δ)	Integration	Multiplicity	Coupling Constant (J, Hz)
18-H	0.27	3H	t	$J_{18,19} = 7.4$, $J_{19\alpha,18} = 7.4$
19α-H	1.17	1H	dd	$J_{19\alpha,19\beta} = 13.6$
19β-H	1.32	1H	dd	$J_{19\beta,18} = 7.4$, $J_{18\beta,19\alpha} = 13.6$
17α-H	1.07	1H	dd	$J_{17\alpha,14} = 5.7$, $J_{17\alpha,17\beta} = 11.3$
17β-H	1.82	1H	dd	$J_{17\beta,14} = 6.4$, $J_{17\beta,17\alpha} = 11.3$
6α-H	1.57	1H	m	–
6β-H	2.15	1H	m	–
3α-H	2.15	1H	m	–
21-H	2.58	1H	bs	–
3β-H	2.96	1H	dd	$J_{3\beta,3\alpha} = 11.6$, $J_{3\beta,14} = 5.2$
5α-H	2.85	1H	m	–
5β-H	3.15	1H	m	–
14-H	3.20	1H	m	–
COOCH_3	3.78	3H	s	–
9-H	7.43	1H	d	$J_{9,10} = 7.7$
10-H	6.97	1H	ddd	$J_{10,9} = 7.7$, $J_{10,11} = 7.0$, $J_{10,12} = 0.9$
11-H	7.09	1H	ddd	$J_{11,10} = 7.0$, $J_{11,12} = 7.9$, $J_{11,9} = 0.8$
12-H	7.29	1H	d	$J_{12,11} = 7.9$
N-H	8.55	1H	s	–
1′-CH_3	0.66	3H	s	–
2′β-H	2.75	1H	m	–
2′α-H	2.85	1H	m	–
3′-H	4.35	1H	dd	$J_{3'2'\alpha} = 11.6$, $J_{3',2'\beta} = 6.0$
4′-CH_3	1.45	3H	s	–
3′-OCCH_3	1.80	3H	s	–
5′-H	5.37	1H	s	–

their upfield chemical shift being consistent with the shielding influence of the carbonyl function at C-3′. The 3′-H resonated at $\delta = 4.35$ as a double doublet ($J_{aa} = 11.6$ Hz, $J_{ae} = 6.0$ Hz), being flanked between olefinic and carbonyl functions. A one-proton singlet at $\delta = 5.37$ was assigned to the 5′-H. The spectrum showed the presence of four protons in the aromatic region. This suggested lack of substitution of the indole chromophore. The indolic N-H proton resonated as a singlet at $\delta = 8.55$.

The spin-spin coupling interactions were established through the COSY-45 spectrum while the multiplicity of the overlapping proton signals were determined from the 2D J-resolved spectrum. The assignment for the C-18 protons at $\delta = 0.27$ could thus be confirmed by its COSY-45 spectrum, which showed strong cross-peaks with the signal at $\delta = 1.17$ (19α-H) and with the signal at $\delta = 1.32$ (19β-H). The C-19α, β protons exhibited both geminal and vicinal couplings in the COSY-45 spectrum. Similarly, the assignments for 17α-H at $\delta = 1.07$ and 17β-H at $\delta = 1.82$ were confirmed by cross-peaks due to geminal coupling as well as vicinal interactions with the 14-H at $\delta = 3.20$. The C-3′ proton at $\delta = 4.35$ showed interactions in the COSY-45 spectrum with 2′α-H ($\delta = 2.85$) and 2′β-H ($\delta = 2.75$). The assignments for the aromatic protons were similarly confirmed from the COSY spectrum. The ^{1}H NMR shift assignments are presented in Table 1.

A two-dimensional nuclear overhauser enhancement spectrum (NOESY) served to establish spatial proximities. The α-stereochemistry of the 21-H proton at $\delta = 2.58$ could be established from the strong cross-peak between 21-H ($\delta = 2.58$) and 6α-H at $\delta = 1.57$. This also indicated that in the preferred conformation of ring C, the 6α-H lies close to 21-H. The NOESY interactions between 21-H and 5′-H also confirmed the α-stereochemistry for the 21-H proton. This could only arise if the five-membered ring is attached at C-15. The C-14 proton at $\delta = 3.20$ showed NOESY interactions with the C-17 protons as well as with the C-19 protons, which could arise if the C-14 proton possessed α-stereochemistry. It also suggested α-stereochemistry for the ester group. If the ester group had been in a β-configuration, the NOESY interactions between 14-H and 17-H protons would then not have been possible. These interactions suggested a catharanthine-type skeleton and also suggested the attachment of the five-membered ring at C-15.

In order to confirm the relative stereochemistry at various asymmetric centres and to record subtle NOE effects not visible in the NOESY spectrum, NOE difference measurements were carried out. Irradiation at $\delta = 0.27$ (18-H) resulted in 5.5% NOE at $\delta = 1.17$ (19α-H) and $\delta = 1.32$ (19β-H). This irradiation also resulted in 5.5% NOE at $\delta = 1.45$ (4′-CH_3), 7.7% NOE at $\delta = 2.58$ (21-H) and 8.9% NOE at $\delta = 5.37$ (5′-H). These NOE interactions served to establish the α-stereochemistry to the C-21 proton and also threw light on the nature of the substituent at C-15. These interactions could have been observed only if the five-membered ring was present at C-15. The NOE interactions between 19β-H, 1′-CH_3 and 5′-H also suggested the presence of a five-membered ring at C-15 without which these interactions would not have been possible. Irradiation at $\delta = 3.20$ (14-H) resulted in 6.5% NOE at $\delta = 3.15$ (5β-H) and 7.9% NOE at $\delta = 4.35$ (3′-H). These NOE interactions could have been observed only if both 21-H and 14-H possessed α-stereochemistry. Irradiation at $\delta = 3.15$ (5β-H) resulted in 15.9% NOE at $\delta = 2.96$ (3β-H) and 3.7% NOE at $\delta = 3.20$ (14-H) suggesting that in the pre-

Table 2. NOE difference measurements on alioline (**1**).

Proton irradiated (δ)	Proton enhanced (δ)	NOE [%]	Proton irradiation (δ)	Proton enhanced (δ)	NOE [%]
3α-H (2.15)	3β-H (2.96)	6.9	17β-H (1.82)	3α-H (2.15)	2.9
	17β-H (1.82)	8.2		5α-H (2.85)	5.7
				17α-H (1.07)	16.2
3β-H (2.96)	5β-H (3.15)	10.2	18-H (0.27)	19α-H (1.17)	5.5
				19β-H (1.32)	5.5
5α-H (2.85)	6α-H (1.57)	3.0		21-H (2.58)	7.7
	6β-H (2.15)	3.4		4′C-H$_3$ (1.45)	5.5
	17α-H (1.07)	2.0		5′-H (5.37)	8.9
5β-H (3.15)	3β-H (2.96)	3.7	19α-H (1.17)	18-H (0.27)	11.2
	5α-H (2.85)	15.7		19β-H (1.32)	9.1
	5β-H (3.15)	4.3		C-CH$_3$ (1.80)	8.9
6α-H (1.57)	6β-H (2.15)	3.9	19β-H (1.32)	18-H (0.27)	4.4
	9-H (7.43)	6.8		19α-H (1.17)	16.2
	21-H (2.58)	15.4		11-CH3 (0.66)	6.2
				5′-H (5.37)	4.9
6β-H (2.15)	5α-H (2.85)	3.6	21-H (2.58)	5′-H (5.37)	7.3
	5β-H (3.15)	15.7		6α-H (1.57)	11.8
	9-H (7.43)	5.2		18-H (0.27)	8.8
	17β-H (1.82)	3.8			
9-H (7.43)	6β-H (2.15)	3.5	1′-CH$_3$ (0.66)	17α-H (1.07)	10.5
	10-H (6.98)	10.5		5′-H (5.37)	4.1
10-H (6.98)	9-H (7.43)	11.8	2′-H (2.80)	3α-H (2.15)	2.5
	11-H (7.09)	6.6		14-H (3.20)	8.9
				3′-H (4.34)	12.2
11-H (7.09)	10-H (6.98)	11.8	3′-H (4.34)	2′β-H (2.75)	4.9
	12-H (7.29)	14.2			
12-H (7.29)	11-H (7.09)	11.2	4′-CH$_3$ (1.45)	18-H (0.27)	4.4
	N-H (8.56)	2.9		5′-H (5.37)	4.9
				C-CH$_3$ (1.80)	3.2
14-H (3.20)	3α-H (2.15)	3.2	5′-H (5.37)	18-H (0.27)	6.5
	3β-H (2.96)	6.9		19β-H (1.32)	3.7
	5β-H (3.15)	6.5			
	3′-H (4.35)	7.9			
	2′α-H (2.85)	4.5			
17α-H (1.07)	1′-CH$_3$ (0.66)	2.4	C-CH$_3$ (1.80)	17α-H (1.07)	3.0
	OC-CH$_3$ (1.80)	8.1		4′-CH$_3$ (1.45)	7.5

ferred conformation of ring C, the C-3β proton lies close to the C-5β proton. The presence of these NOE interactions and the absence of any interaction of these protons with the C-16 ester methyl protons suggested an α-stereochemistry of the ester group. Similarly, irradiation at $\delta = 5.37$ (5′-H) resulted in 6.5% NOE at $\delta = 0.27$ (C-18H), suggesting the proximity of the five-membered substituent to the ethyl side chain. The NOE results are summarized in Table 2.

The ^{13}C NMR spectrum ($CDCl_3$, 75 MHz) showed the presence of thirty carbon atoms. The multiplicity assignments were made by carrying out DEPT measurements with the last polarization pulse angle set at 45, 90 and 135°, and the assignments were confirmed by heteronuclear correlated spectroscopy [5]. The methyl carbon of the ethyl side chain resonated at $\delta = 8.31$ while the methylene carbon appeared at $\delta = 36.85$. The carbon atom C-21 appeared at $\delta = 63.23$, its upfield chemical shift suggesting α-stereochemistry [19, 20]. The C-1′ methyl and acetyl methyl carbons of the five-membered ring resonated at $\delta = 28.31$ and 22.32, respectively. The 4′-methyl appeared at $\delta = 21.41$ indicating that the methyl group was attached to the olefinic carbon. The signal for C-3 appeared at $\delta = 54.10$ while C-5 resonated at $\delta = 47.98$, the downfield chemical shifts reflecting the presence of a nitrogen function at α position to these carbons. The quaternary carbon signal at $\delta = 205.68$ was assigned to the ketonic carbonyl on the five-membered ring while the ester methyl and its carbonyl carbon resonated at $\delta = 52.46$ and 174.31, respectively. The ^{13}C NMR

Table 3. ^{13}C NMR shift assignments for alioline **(1)**.

Carbon No.	Chemical shift (δ)	Carbon No.	Chemical shift (δ)
2	134.79	18	8.31
3	54.10	19	36.85
5	47.89	20	136.15
6	21.99	21	63.23
7	111.23	$COOCH_3$	52.46
8	127.45	$COOCH_3$	174.31
9	121.88	$1'$	37.34
10	119.12	$2'$	22.52
11	119.00	$3'$	40.66
12	110.65	$4'$	135.75
13	142.60	$5'$	130.24
14	49.88	$1'$-CH_3	28.31
15	130.81	$3'$-$OCCH_3$	22.32
16	45.33	$3'$-$OCCH_3$	205.68
17	47.30	$4'$-CH3	21.41

shift assignments of alioline (**1**) are presented in Table 3.

Hetero-COSY experiments [11] were carried out to identify the relationship between carbons and their respective protons. The methyl carbon signal at $\delta = 8.31$ showed a cross-peak with the methyl protons signal at $\delta = 0.27$ in the hetero-COSY spectrum. Carbon-17 ($\delta = 47.30$) showed cross-peaks with the two proton signals at $\delta = 1.07$ (17α-H) and 1.82 (17β-H), thereby indicating non-equivalence of the C-17 protons. The assignments of the C-3′ carbon ($\delta = 40.67$) and the attached proton at $\delta = 4.34$ were confirmed by the cross-peak in the hetero-COSY spectrum. The C-3 signal at $\delta = 54.11$ showed cross-peaks with the proton signals at $\delta = 2.15$ (3α-H) and 2.96 (3β-H). Similarly, the C-21 carbon ($\delta = 63.22$) showed a cross-peak with the proton signal at $\delta = 2.58$. The olefinic carbon signal at $\delta = 130.24$ showed a cross-peak with the proton signal at $\delta = 5.37$. The assignments of the aromatic protons were also confirmed by the hetero-COSY spectrum.

Experimental Section

Instrumentation: UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded on a JASCO A-302 spectrophotometer. High-resolution mass spectra were recorded on a Varian MAT-312 mass spectrometer connected to a PDP 11/34 (DEC) computer system. The ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AM-300 NMR spectrometer. The ^{13}C NMR spectra were recorded at 75 MHz on the same instrument. The optical rotation was recorded with a Polartronic Universal Australian Standard K-157 digital polarimeter. TLC experiments were performed on silica gel plates (GF–254, 0.2 mm, E. Merck).

*Isolation of alioline (**1**):* The ethanolic extract of dried leaves of *Catharanthus roseus* was concentrated on a rotary evaporator under reduced pressure. The concentrate was acidified with 2% tartaric acid. The material thus obtained was successively basified with ammonia and extracted with chloroform. The pH∼3 fraction was subjected to silica-gel column chromatography. The column was eluted with increasing polarities of petroleum ether/chloroform. The fraction obtained with petroleum ether/chloroform (6.0:2.0) was dried and further subjected to the PTLC on precoated silica-gel (GF–254) plates with petroleum ether/chloroform (7.5:2.5) as the solvent system. This resulted in the isolation of a pure compound, alioline (**1**) as an amorphous material (25 mg, $Rf = 0.23$, 1.25×10^{-4}% yield).

Spectral data: $[\alpha]_D^{24} + 105.5^\circ$ (MeOH). UV (MeOH): λ_{max} ($\log\varepsilon$) = 227 (4.13), 283 (3.69) and 292 nm (3.61). IR ($CHCl_3$): $\nu_{max} = 2910$ (C-H) 3130 (N-H), 1720 (ester C=O), 1680 (ketonic C=O), 1600 (C=C), 750 cm^{-1} (aromatic C-H). HRMS: $m/z = 472.2720$ ($C_{30}H_{36}N_2O_3$, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz): see Table 1; NOED (%): Table 2. ^{13}C NMR ($CDCl_3$, 75 MHz): Table 3.

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