JAMTINE-N-OXIDE - A NEW ISOQUINOLINE ALKALOID FROM COCCULUS HIRSUTUS

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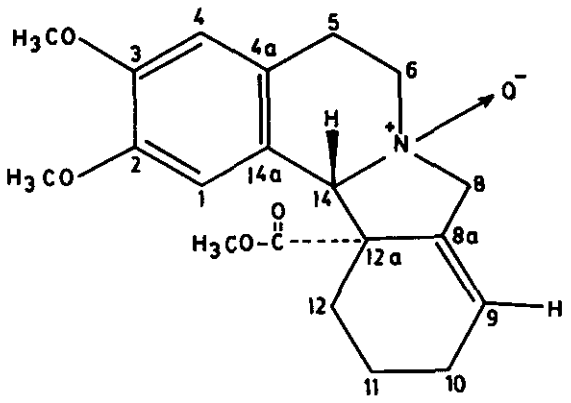
Abstract - A new isoquinoline alkaloid, jamtine-N-oxide has been isolated from Cocculus hirsutus. Its structure has been assigned as $C_{20}H_{25}NO_5$ (1) on the basis of spectral studies. Its stereochemistry has been determined by homonuclear 2D ^{1}H-NMR (COSY-45, J-resolved, NOESY) and ^{13}C-NMR assignments made by GASPE experiments.

Cocculus hirsutus (Syn: C.villosus) DC (Menispermaceae), locally called Jamti-ki-bel, is a climbing shrub and is commonly found in Karachi, Sind and Kutch. Its various parts are highly reputed for their medicinal properties in the indigenous system of medicine[1-4]. Earlier investigations on various parts of this plant led to the isolation of trilobine, isotrilobine, coclaurine, magnoflorine and sitosterol[4-6]. As a result of our continuing investigations on leaves of Cocculus hirsutus, we nave isolated a new alkaloid, to which structure (1) has been assigned on the basis of extensive NMR studies including homonuclear 2D ^{1}H-NMR (COSY-45, J-resolved, NOESY), ^{13}C-NMR and GASPE experiments[7-10].

RESULTS AND DISCUSSION

A pure alkaloid Jamtine-N-oxide (1) was isolated as a gum from Cocculus hirsutus as described in experimental. Its UV spectrum showed absorptions at 220, 239 and 300 nm. The IR spectrum indicated the presence of an ester carbonyl at 1720 cm^{-1}. High resolution mass spectrum (HRMS) of the alkaloid afforded the molecular ion peak at m/z 359.1763, corresponding to the molecular formula $C_{20}H_{25}NO_5$, indicating nine degree of unsaturation in the molecule. Other prominent peaks were found to occur at m/z 343, 312, 298, 285, 252, 226 and 208. The peak at m/z 343 ($C_{20}H_{25}NO_4$) and m/z ($C_{19}H_{22}NO_3$) corresponded to the loss of 16 m.u. (oxygen) and methoxy from the molecular ion and m/z 343, respectively. The base ion peak at m/z 285 represented the loss of 58 m.u. carbomethoxy from m/z 343 and suggested the attachment of the methyl ester to a quaternary carbon[11]. The molecular ion was certainly confirmed by FAB mass spectrometry[12].



(1)

The ^{1}H-NMR spectrum of jamtine-N-oxide (1) ($CDCl_3$, 300 MHz) showed a double doublet at δ 1.85 ($J_{5\beta,5\alpha}$=11.6Hz, $J_{5\beta,6\alpha}$= $J_{5\beta,6\beta}$= 4.6Hz) assigned to the C-5βproton. The C-5α proton resonated as a double doublet at δ1.68 ($J_{5\alpha,5\beta}$= 11.6 Hz, $J_{5\alpha,6\alpha}$= $J_{5\alpha,6\beta}$= 5.3Hz). The two protons at C-6 resonated at δ3.66 ~ 3.90 as a multiplet, while a singlet at δ 4.12 was assigned to the C-14 proton. The C-8α proton resonated at δ 3.53 as a doublet ($J_{8\alpha,8\beta}$=10.0Hz) while the C-8β proton appeared at δ4.32 as a doublet ($J_{8\beta,8\alpha}$=10.0Hz). The rather downfield chemical shift for the C-8α and C-14 protons is also due to the N^+ function at α-disposition. The rather upfield singlet at δ3.25 was assigned to the carbomethoxy group, which is due to the shielding influence of the aromatic nucleus[13,15]. The C-9 olefinic proton appeared at δ 5.96 as a broad singlet. The C-4 proton resonated at δ 7.53 as a singlet while another singlet at δ 6.77 was assigned to the C-1 proton. The signals of aromatic protons at δ 6.77 and 7.53 as singlet shows due to 2,3-disubstituted benzene ring system, which indicates that the two methoxy signals at δ 3.89 must be located at C-2 and C-3.

Two dimensional ^{1}H-NMR measurements were carried out to verify the assignments. The coupling interactions were established through correlated spectroscopy (COSY-45) spectrum while the multiplicity of the overlapping proton signals was determined from the 2D-J-resolved spectrum[7-10]. The assignments for the C-10 protons at δ2.70 could thus be confirmed by its COSY-45 spectrum, which showed strong cross peak with the signal at δ 5.96 for the C-9 olefinic proton. The assignments for the C-5β proton at δ 1.85 and C-5α proton at δ 1.68 was also confirmed by the COSY-45 interactions with the C-6 protons at δ 3.68 and δ 3.90, respectively. The C-8α proton at δ3.53 showed a strong COSY interaction with the C-8β proton at δ4.32.

The Nuclear Overhauser enhancement spectroscopy (NOESY) spectrum served to established the spatial proximities. The stereochemistry of the ester group could be confirmed from the NOESY spectrum since it showed strong cross peaks with the signals at δ 6.77 for the C-1 proton and at δ3.89 for the methoxy proton. This could only arise it the ester group possessed α-stereochemistry, which would also result in C-14 proton having a β-configuration. The NOESY interactions between C-14 proton and C-5β proton also confirmed the βstereochemistry of the C-14 proton.

The multiplicity assignments in the ^{13}C-NMR spectrum ($CDCl_3$, 75 MHz, Table-I) were made by carrying out gated spin echo (GASPE) experiments. The methyl carbon of the ester resonated at δ52.31 while the two methoxy carbons appeared at δ 55.94 and δ 56.25, respectively. The C-9 olefinic carbon resonated at δ131.09 while the C-1 and C-4 carbons appeared at δ 112.40 and δ124.40, respectively. The signal at δ 80.71 was assigned to the C-12a the rather down field chemical shift to this carbon may be due to the α-disposition double bond and β-N^+ function. The C-14 carbon resonated at δ 72.88 while the C-6 and C-8 carbons appears at δ 56.46 and δ62.25, respectively. The rather down field chemical shifts of these carbons reflect the presence of the α-quaternary nitrogen.

Table-I: ^{13}C-NMR chemical shifts of jamtine-N-oxide (1)

Carbon No.	(δ)	Carbon No.	(δ)
1	112.40	10	22.40
2	158.78	11	25.26
3	158.78	12	27.14
4	124.40	12a	80.71
4a	135.20$^+$	14	72.88
5	31.18	14a	134.58$^+$
6	56.42	2-O$\underline{C}$H$_3$	56.25*
8	62.23	2-O$\underline{C}$H$_3$	55.94*
8a	134.34$^+$	$\overset{O}{\overset{\|}{C}}$-O$\underline{C}H_3$	52.31
9	131.09	$\underline{\overset{O}{\overset{\|}{C}}}$-OCH$_3$	172.60

*+ The values marked with identical symbols are interchangeable.

** Weak signal.

Reduction of jamtine-N-oxide (1) in dichloromethane with phosphorous trichloride led to deoxygenation of the N-oxide (1) to afford a faster running product. The $N^+ \rightarrow O^-$ absorptions disappeared in the IR spectrum and the mass spectrum of the deoxygenated product afforded the M^+ 343, which was confirmed by FAB mass spectrometry.

In order to ascertain that jamtine-N-oxide is a genuine natural product and not an artifact of isolation, the deoxygenated product was separately exposed to identical extraction and separation procedure but no conversion to the corresponding N-oxide was discernible. Further confirmation of this was obtained by detection of jamtine-N-oxide (1) by TLC in freshly prepared crude extracts.

EXPERIMENTAL

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and IR spectra were recorded on JASCO A-302 spectrophotometer. HRMS were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 (DEC) computer system. NMR spectra were recorded at 300 MHz in $CDCl_3$ on a Bruker AM-300 NMR spectrometer. TLC experiments were performed on silica gel (GF-254, 0.2 mm) plates (E.Merck).

Isolation of Jamtine-N-oxide (1): The plant material (20 kg) were collected from the Karachi region and was identified by the plant Texonomist, Department of Botany, University of Karachi where a voucher specimen is deposited. The plant material was chopped into small pieces and extracted exhausively with EtOH. The ethanolic extract was evaporated under reduced pressure. The material thus obtained was extracted with EtOAc. The aqueous layer was basified with ammonia and the crude alkaloids were extracted with chloroform. The chloroform layer was evaporated and dried with Na_2SO_4 (anhydrous). This was extracted with hexane, ether, chloroform and methanol. The ether-soluble portion was subjected to preparative TLC on silica gel. (GF-254) precoated plates with chloroform:methanol (1:1)as the solvent system. The major alkaloid, jamtine-N-oxide (1) (Rf=0.3) 18 mg. was separated as a viscus oil.

UV : (CH_3OH) λ_{max}, 220, 239 and 300 nm, λ_{min} 237 and 265 nm. IR : ($CHCl_3$) ν_{max}, 1720 cm^{-1} (ester carbonyl), 1180-1280 cm^{-1} ($N^+ \rightarrow O^-$) HRMS : M^+ 359.1763 ($C_{20}H_{25}NO_5$, 4%), 343.1778 ($C_{20}H_{25}NO_4$, 4%) 312.1596 ($C_{19}H_{22}NO_3$, 18%), 298.1439 ($C_{18}H_{20}NO_3$, 8%) 285.1723 ($C_{18}H_{23}NO_2$, 100%), 252.1027 ($C_{16}H_{14}NO_2$, 68%) 226.1221 ($C_{15}H_{16}NO$, 30%), 208.0792 ($C_{14}H_{10}NO$, 20%).

^{1}H-NMR : ($CDCl_3$, 300 MHz, δ ppm) : 1.68 (1H, dd, $J_{5\alpha,5\beta}$=11.6Hz, $J_{5\alpha,6}$ = 5.3Hz, C-5αH), 1.85 (1H, dd, $J_{5\beta,5\alpha}$=11.6Hz, $J_{5\beta,6}$ = 4.6Hz, C-5βH), 1.90 (1H, m, C-11H), 2.28 (1H, m, C-11H), 2.4 (1H, m, C-12H), 2.70 (2H, m, C-10H), 3.25 (3H, s $-\overset{O}{\overset{\|}{C}}-OCH_3$), 3.53 (1H, m, C-12H), 3.53 (1H, d, $J_{8\alpha,8\beta}$= 10.0 Hz, C-8 α H), 3.66 (1H, m, C-6H), 3.89 (6H, s, 2(O-CH_3)), 3.90 (1H, m, C-6H), 4.12 (1H, s, C-14H), 4.32 (1H, d, $J_{8\beta,8\alpha}$=10.0 Hz, C-8βH), 5.96 (1H, bs, C-9H), 6.77 (1H, s, C-1H), 7.53 (1H, s, C-4H), ^{13}C-NMR ($CDCl_3$, 75MHz) (ppm) : Table-I

Deoxygenation of Jamtine-N-oxide (1)

Jamtine-N-oxide (1) (2 mg) was dissolved in CH_2Cl_2 0.5 ml and PCl_3 0.1 ml. The mixture was stirred for 30 min at 30°C. Basification with aqueous NH_3 and extraction with $CHCl_3$ afforded an amorphous material which was identified as deoxyjamtine-N-oxide by study of its IR absorptions mass, and Rf value.

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