STEREOSTRUCTURE OF SUBULACINE-N-OXIDE: A NEW PYRROLIZIDINE ALKALOID FROM HELIOTROPIUM SUBULATUM

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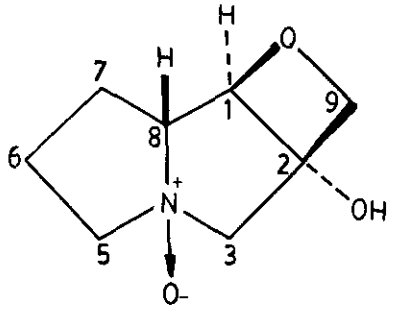
Abstract- A new pyrrolizidine alkaloid, subulacine-N-oxide has been isolated from Heliotropium subulatum. Its structure and stereochemistry has been assigned as (1) on the basis of spectral studies including 2D-nmr.

Heliotropium species (Boragenaceae) constitute a rich source of pyrrolizidine alkaloids some of which possess antitumor, carcinogenic and hepatotoxic activities[1-2]. The wide range of biological activities exhibited by these alkaloids prompted us to carry out systematic chemical studies on various local plants of the genus Heliotropium. Among these Heliotropium subulatum is an erect scabrous annual shrub[3] widely distributed in Pakistan, the north western India and in Africa. Its various parts are highly reputed for their medicinal properties in the indigenous system of medicine[4]. Literature survey shows that no phytochemical studies have so far been carried out on this species. As a result of our investigation on the fresh and undried plant material of Heliotropium subulatum, we have isolated a new pyrrolizidine alkaloid, to which stereo-structure (1) has been assigned on the basis of extensive nmr studies including homonuclear 2D ^{1}H-nmr (COSY-45, J-resolved, NOESY), heteronuclear ^{1}H-^{13}C correlated spectroscopy, ^{13}C-nmr and DEPT experiments.

RESULTS AND DISCUSSION

Subulacine-N-oxide was isolated as a viscous oil, $[\alpha]_D^{20}$ - 110.2° (c=0.2, $CHCl_3$). Its uv spectrum showed absorption at 205 nm. The ir spectrum indicated the presence of OH group at 3400 cm^{-1} and N^+-O^- absorption at 1180-1280 cm^{-1}. The resistance to acetylation and oxidation revealed the tertiary nature of the hydroxyl group. The high resolution mass spectrum (hrms) gave the molecular ion peak at m/z 171.089530, corresponding to the molecular formula $C_8H_{13}NO_3$, indicating three degree of unsaturation in the molecule. Other prominent peaks were found to occur at m/z 155, 138, 124, 108, 96, 83, 82, 80, 70 and 55. The peak at m/z 155 ($C_8H_{13}NO_2$) corresponds to the loss of 16 m.u. (oxygen). The peaks at m/z 138 ($C_8H_{12}NO$) and m/z 124 ($C_7H_{10}NO$) represent the loss of OH and CH_2OH from the ion m/z 155. Further peaks at m/z 83, 82, 80 and

the base peak at m/z 70 are characteristic of pyrrolizidine alkaloids in which the ring A has no substituent[5]. The molecular ion was further confirmed by FD mass spectrometry.



(1)

The ^{1}H- nmr spectrum of subulacine-N-oxide ($CDCl_3$, 400MHz) showed a broad singlet at δ 5.2 which disappeared on shaking with D_2O and could be assigned to OH group. The most downfield double doublet at δ 3.77 ($J_{9\beta,9\alpha}$ = 12.7Hz) could be assigned to the nonequivalent C-9 methylene protons while H-8β resonated as triplet at δ 3.48 ($J_{8\beta,7\alpha} = J_{8\beta,7\alpha}$ = 7Hz). The H-3α appeared as doublet at δ 3.03 ($J_{3\beta,3\alpha}$ = 12.5Hz) and another doublet of the same coupling constant at δ 2.89 was assigned to H-3β[6]. Both the protons at C-5 gave multiplets; the one at lower field at δ 2.93 was assigned to H-5α while the upfield multiplet at δ 2.54 could be assigned to H-5β , according to the earlier findings of Culvenor et al[6]. The H-6α and H-6β resonated as multiplets at δ 1.80 and δ 1.65, respectively. Further multiplets at δ 1.77 and δ 1.70 corresponded to H-7α and H-7β while the broad singlet at δ 3.55 was assigned to H-1φ. All the proton assignments were confirmed by decoupling experiments. Irradiation at the frequency of H-3α (or H-3β) caused the doublet of H-3β (or H-3α) to collapse into singlets. Irradiation at the frequency of H-6α (or H-6β) collapsed the multiplets of H-5α and H-5β to broad quartets, and simplified the multiplets of H-7α and H-7β. On the other hand, irradiation at the frequency of H-7α (or H-7β) caused the triplet of H-8β at δ 3.48 to collapse into doublet (J = 7Hz) while irradiation at δ 3.48 simplified the multiplets of H-7α and H-7β at δ 1.77 and δ 1.70, respectively. The irradiations at δ 3.55 and δ 3.77 has no effect on rest of the spectrum confirming the non interaction of H-1α and C-9 protons with other protons in the molecule. The ^{13}C- nmr spectrum ($CDCl_3$, 75MHz) showed eight carbon

atoms. The multiplicity was determined by using DEPT experiments with the polarization pulse O=45°, 90° and 135°. The experiments revealed five methylene, two methine groups and a quaternary carbon. The assignments shown in Table-1 are in accordance with those of Mody et al[7].

Table-I: ^{13}C-Nmr chemical shifts of subulacine-N-oxide (1).

Carbon No.	δ	Carbon No.	δ
1	64.401	6	26.889
2	71.98	7	25.261
3	53.639	8	63.636
5	56.309	9	59.782

The structure of subulacine-N-oxide was further corroborated by two dimensional ^{1}H-nmr measurements. The chemical shifts of various protons were authenticated through heteronuclear ^{1}H-^{13}C correlated spectroscopy (Heterocosy)[8]. The signals of C-1, C-3, C-5, C-8 and C-9 could easily be correlated with chemical shifts of their respective protons. The exact chemical shift, multiplicities and coupling constants were also confirmed from the 2D-J-resolved spectrum[8]. The coupling interactions were established by homonuclear ^{1}H-^{1}H correlated spectroscopy (COSY-45) which showed connectivity of H-8 at δ 3.48 with both the protons at C-7. The H-5α and H-5β showed cross peaks with H-6α and H-6β at δ 1.65 and δ 1.80. The protons at C-3 and also at C-9 showed connectivity only with each other, respectively.

The stereochemistry of subulacine-N-oxide (**1**) was derived with the help of ^{1}H-nmr parameters of 1α,2α-epoxy-1β-hydroxymethyl-8α-pyrrolizidine (**2**), prepared by epoxidation of supinidine with perbenzoic acid[6]. Comparing the chemical shifts of the C-3 protons in (**1**) and (**2**) with those of the corresponding protons in platynecine and retronecine[6], it is seen that H-3β in (**1**) and H-3α in (**2**) are at abnormally low field. The additional deshielding must be due to the oxide ring. The C-3 proton which is cis to the oxide ring is not located opposite to its face where shielding occurs[9]. If the influence of the ring on the C-3 protons is due to electric dipole effects, it seems likely that it is the cis proton which is deshielded. The chemical shift of H-3β in (**1**) is very close to that of the corresponding proton in naturally occurring 1β,2β-epoxy-1α-hydroxymethyl-8α-pyrrolizidine (**3**), providing evidence for the β configuration of four membered oxide ring and

the α-configuration of hydroxyl group at C-2. Finally the configuration of H-8 was established as β by the absence of coupling with H-1α. The dihedral angle between these protons is almost 90° and hence no coupling is observed in confirmity to the earlier findings of Karplus[10]. Conclusive evidence for the stereo-structure was provided by establishing spatial proximities through the Nuclear Overhauser enhancement spectroscopy (NOESY). The NOESY interaction between H-8, H-7β and H-6β confirmed the β stereochemistry of the C-8 proton. The stereochemistry of the hydroxyl group could also be confirmed since it showed strong cross peak with the signal at δ3.55 for the H-1α.

Reduction of (1) in dichloromethane with phosphorous trichloride led to deoxygenation of the N-oxide to afford subulacine as faster running product. The mass spectrum gave molecular ion peak at m/z 155, which was confirmed by FD mass spectrometry. The $N^+—O^-$ absorption also disappeared in the ir spectrum.

In order to ascertain that subulacine-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 subulacine-N-oxide by TLC in freshly prepared crude extracts.

EXPERIMENTAL

Uv spectrum was 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 on a Bruker AM-400 spectrometer. TLC experiments were performed on silica gel (PF-254, 0.2 mm) plates (E.Merck).

Isolation of Subulacine-N-oxide (1): The fresh plant material (40 kg) were collected from the Karachi region and was identified by the plant Taxanomist, Department of Botany, University of Karachi where a voucher specimen is deposited. The plant material was chopped into small pieces and extracted with EtOH. The ethanolic extract was evaporated under reduced pressure and the material thus obtained was partitioned between water and EtOAc. The aqueous layer was basified with ammonia and the liberated crude alkaloids were extracted with chloroform. The residue recovered from the chloroform layer was chromatographed over silica gel and eluted successively with hexane, chloroform and methanol. The chloroform-soluble portion was subjected to preparative TLC using chloroform:methanol (8:2) as the solvent system. The major alkaloid, subulacine-N-oxide (1) (Rf=0.3) 20 mg. was separated as a viscous oil.

Uv: (CH_3OH) λ_{max}, 205 nm. Ir: ($CHCl_3$) ν_{max}, 3450 cm^{-1} (OH), 1180-1280 cm^{-1} ($N^+—O^-$). HRMS: M^+ 171.08953 ($C_8H_{13}NO_3$, 1.5%), 155.09446 ($C_8H_{13}NO_2$, 22.8%), 138.09204 ($C_8H_{12}NO$, 1.58%), 124.07631 ($C_7H_{10}NO$, 19.87%), 96.045173 (C_5H_6NO, 13.54%), 83 (3%), 82 (4%), 80 (39%), 70.065670 (C_4H_8N, 100%). ^{1}H-Nmr : ($CDCl_3$, 400MHz, δ ppm): 1.65 (1H,m,6-Hβ), 1.70 (1H,m,7-Hβ), 1.77 (1H,m,7-Hα), 1.80 (1H,m,6-Hα), 2.54 (1H,m,5-Hβ), 2.89 (1H,d,$J_{3\beta,3\alpha}$ = 12.5Hz, 3-Hβ), 2.93 (1H,m,5-Hα), 3.03 (1H,d, $J_{3\alpha,3\beta}$ = 12.5Hz, 3-Hα), 3.48 (1H,t,$J_{8\beta,7\alpha} = J_{8\beta,7\beta}$ = 7Hz, 8-Hβ), 3.55 (1H,brs, 1-Hα), 3.77 (2H,dd, $J_{9\alpha,9\beta}$ = 12.7Hz, 9-H_2). ^{13}C-Nmr ($CDCl_3$, 75MHz, δ ppm): Table-1.

Deoxygenation of Subulacine-N-oxide (1): Subulacine-N-oxide (3 mg) was dissolved in CH_2Cl_2 0.5 ml and PCl_3 0.1 ml. The mixture was stirred for 1h at 30°C. Basification with ammonia and extraction with chloroform afforded a gummy residue which was identified as deoxysubulacine-N-oxide (subulacine) by its IR, mass and Rf value.

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