ANDRACHAMINE A NEW ALKALOID FROM *ANDRACHNE ASPERA*

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Abstract- A new piperidine alkaloid andrachamine was isolated from the medicinal plant Andrachne aspera. Its structure was established by spectroscopic studies.

The plant Andrachne aspera Spreng (Euphorbiaceae) is a small undershrub, found commonly in arid planes of Sind and Baluchistan[1]. It is reported to have medicinal importance in indigenous system of medicine specially for sore eyes and to improve eye sight[2,3]. The crude alkaloidal mixture is reported to possess biological and antibacterial activity in various biological assays. The plant is also described as a true substitute of Polygala senega in earlier literature[5], although *this property was disputed later by another group*[7].

In the present communication we wish to report the isolation and structure elucidation of a new piperidine alkaloid andrachamine (Fig.1) from the above plant.

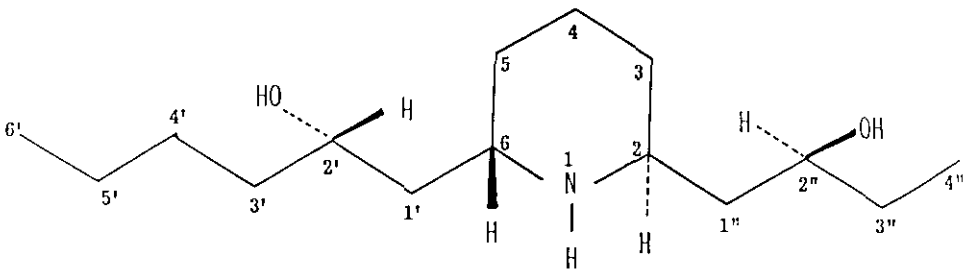

Figure-1

Andrachamine was obtained from crude alkaloidal mixture of the plant as described in the Experimental.

In the high resolution ms the molecular ion appeared at m/z 257.23461 which corresponds to $C_{15}H_{31}NO_2$ (calc. 257.235466). The ir spectrum ($CHCl_3$) exhibited a broad band at 3200-3600 cm^{-1} showing the presence of -OH group. The uv (MeOH) spectrum does not show any significant absorption except an end absorption at 220 nm.

The ^1H nmr (300 MHz $CDCl_3$) revealed a signal centered at δ 0.91 which is due to two overlapping triplets with integration of 6 protons, attributed to the presence of two methyl groups adjacent to

the methylene protons.

A broad signal at δ 3.85 (2H), suggested the presence of two protons geminal to -OH groups. Another broad signal centered at δ 3.20 (2H) could be assigned to the protons at 2 and 6 position of piperidine ring. This also led to the conclusion that the alkaloid has a disubstituted piperidine ring, which was conformed by the peak at m/z 82.0654 corresponding to C_5H_8NO (calcd. 82.0656) and ^{13}C assignment at δ 59.05, 40.06, 18.7 corresponding to $C_{2/6}$, $C_{3/5}$ and C_4 of 2,6, disubstituted piperidine ring respectively. On irradiation at δ 3.20 and δ 3.85 we observed no coupling between these two signal and this was confirmed by COSY 45 experiment. This leads to the conclusion that there are no -OH groups at carbon atoms adjacent to C_2 and C_6 of piperidine rings and hence protons geminal to -OH are coupled to methylenic protons only.

The E.I. Mass spectra (70 eV) exhibited M^+ at m/z 257 and other important fragments were at m/z 242, 227, 214, 200, 197, 184, 170, 156 and 82.

The fragments at m/z 242, 227, 214, 200, 170 and 156 are indicative of loss of CH_3, C_2H_5, C_3H_7, C_4H_9, $C_5H_{11}O$ and $C_6H_{13}O$ (CH_3-$(CH_2)_3$-CHOH-CH_2) from the molecular ion.

On the other hand fragments at m/z 198 and 184 show loss of C_3H_7O (CH_3-CH_2-CHOH) and C_4H_9O (CH_3-CH_2-CHOH-CH_2) from the molecular ion (Fig.2). The elemental composition of these fragments was confirmed by peak matching.

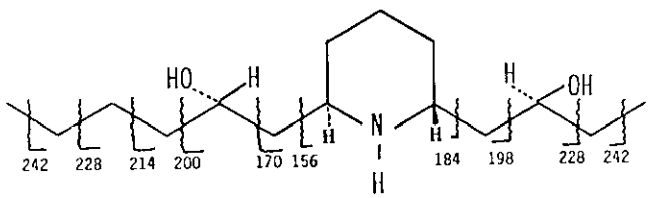

Figure-2

This observation leads to the conclusion that one substituent chain has four and the other six carbon atoms both having one OH group at the carbon next to the one attached to the piperidine ring. The alkyl chains are attached to the ring at 2 and 6 position of piperidine. The trans configuration at both substituents at C_2 and C_6 position was confirmed by comparing ^{13}C chemical shift of C_4 with previously reported ^{13}C values of 2,6-disubstituted piperidines[6]. The observed value for C_4 of trans 2,6-disubstituted piperidine is 18.7 (reported is 19.5) where as for cis isomer is 25.1 ppm. The assigned ^{13}C (75 MHz, $CDCl_3$) values are δ C_2 59.06, C_3 40.06 C_4 18.7, C_5 40.06, C_6 59.06, $C_{1'}$ 31.0, $C_{2'}$71.0, $C_{3'}$ 39.6, $C_{4'}$ 29.4, $C_{5'}$ 23.0, $C_{6'}$ 13.9, $C_{1''}$ 40.3, $C_{2''}$ 73.6, $C_{3''}$ 29.4, $C_{4''}$ 9.9.

Application of Horeau's method led to the isolation of (+) phenylbutyric acid, if we assume that

the (±) phenylebutyric anhydride reacts with the OH groups at 2' and 2" positions of alkyl side chains with equal optical yields due to similar environment then it may be concluded that the absolute configuration at both these centers is S.

EXPERIMENTAL

The ^{1}H and ^{13}C nmr were recorded in $CDCl_3$ using $CHCl_3$ as internal standard with a Bruker AM-300 spectrometer. The uv spectra was measured in MeOH with Shimadzu UC-240 Graphicord spectrometer. The ir spectrum was scanned in $CDCl_3$ on a Jasco IRA-1 spectrometer. The mass spectra were recorded on Finnigan MAT 312 double focusing mass spectrometer linked with PDP 11/34 computer.

The plant Andrachne aspera was collected from hills near Karachi University campus and identified by plant taxonomist of Department of Botany. A voucher specimen is deposited in the Herbarium of Department of Botany, University of Karachi.

The purity of the alkaloid was checked on tlc plate Si-60 0.25 mm (RDH) using 8:2, 9:1 and 8.5:1.5 $CHCl_3$ MeOH as mobile phase and alkaloids were detected by Dragendorf's reagent.

Isolation was carried out as follows: Fresh plant Andrachne aspera was homogenised in EtOH and the filtrate obtained was evaporated under reduced pressure and the residue obtained was partitioned between ethyl acetate and H_2O (6 litre). The aqueous portion was basified to pH 9.0 by adding ammonia and extracted with chloroform (6 litre). On evaporating chloroform under reduced pressure a crude alkaloidal mixture was obtained. This crude alkaloidal mixture was submitted to Flash chromatography using Si-60 as stationary phase and $CHCl_3$-MeOH (8:2) as mobile phase. This gave 5 fractions. Fraction II was further chromatographed on flash chromatograph using $CHCl_3$-MeOH (8.5:1.5) as mobile phase, yielding 3 more fractions.

Fraction 2 of this step was further chromatographed by low pressure liquid chromatography, using Lobar column Lichroprep Si-60 (40-63 μm) (Merck) and $CHCl_3$-MeOH (8.5:1.5) as mobile phase.

Four fractions were thus obtained; the fraction 3 was alkaloidal, showed two spots on tlc and was finally separated by HPLC using straight phase column with $CHCl_3$-MeOH (9.5:0.5) as mobile phase with flow rate 2ml/min. This provided andrachamine as very hygroscopic needles in pure form. $[\alpha]^{25}=-74°$ ($CHCl_3$ c=2.0) E.I. mass (70 ev) M^+ 257 other important fragments are at m/z 242 (M^+-CH_3), 227 ($M^+-C_2H_5$) 214 ($M^+-C_3H_7$), 200 ($M^+-C_4H_9$) 198 ($M^+-C_3H_7O$) 184 ($M^+-C_4H_9O$), 170 ($M^+-C_5H_{11}O$) 156 ($M^+-C_6H_{13}O$).

APPLICATION OF HOREAU'S METHOD:

To a solution of andrachamine 4 mg in about 0.2 ml of dry pyridine 2 molar equivalents of (±)-phenylbutanoic anhydride were added and the reaction mixture was allowed to stand for 1 h at room temperature. Water (about 2.5 ml) was added and allowed to stand for 3 h at room

temperature. The mixture was basified with 0.1N NaOH and extracted with $CHCl_3$ to remove the ester formed. The aqueous phase is then acidified with 1N HCl and extracted with benzene. The extract was dried over anhydrous sodium sulphate and filtered and the volume made to 2 ml, reading at D line afforded + 0.899°.

ACKNOWLEDGEMENT

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