HETEROCYCLES, Vol 22, No. 9, 1984

THE ISOLATION AND STRUCTURE OF STRICTIMINE, A NEW PIPERIDINE ALKALOID FROM THE ROOTS OF *RHAZYA STRICTA*

Atta-ur-Rahman* and Khurshid Zaman
H.E.J.Research Institute of Chemistry
University of Karachi, Karachi-32/Pakistan

Abstract — A new bispiperidine alkaloid, "strictimine", has been isolated from the roots of *Rhazya stricta* to which structure (1) has been assigned on the basis of spectroscopic studies.

Rhazya stricta Decaisne (Apocynaceae) is abundantly distributed in various regions of Pakistan. It is a small glabrous, erect shrub growing in the north west region of the subcontinent. *Rhazya stricta* is reputed as curative for chronic rheumatism[1-4] and for its anticancer activity.[5-7] While much work has been carried out on the isolation of alkaloids from the leaves and seeds of *Rhazya stricta*, the roots have been little investigated. As a result of studies carried out on its roots, we have isolated a new alkaloid which has been identified as (1) by spectroscopic studies.

Powdered *R.stricta* roots (75 kg) were percolated with ethanol. The ethanolic extracts were concentrated to a dark brown gum. The crude extract was acidified with 10% acetic acid and the acidic solution was washed with petroleum ether (60-80°). The aqueous layer was extracted with chloroform to remove the non-alkaloidal components, basified with ammonia and the liberated alkaloids extracted first with chloroform and then with ethylacetate. The chloroform and ethylacetate extracts were combined, dried (anhydrous Na_2SO_4), filtered and evaporated to afford the crude alkaloids (145 gm). These were dissolved in tartaric acid solution and extracted with chloroform at different pH values. The pH of the aqueous solution was adjusted to 2.5 with ammonia and the solution extracted with chloroform. The chloroform extracts were dried (anhydrous Na_2SO_4), filtered and concentrated *in vacuo* to afford the crude alkaloids (40 gm).

These were subjected to column chromatography on a silica gel column. Elution first with increasing polarities of petroleum ether-ethylacetate, ethylacetate, ethylacetate-methanol and finally with methanol gave various fractions of alkaloids. The fraction eluted with petroleum ether : ethylacetate (6.5:3.5) contained two alkaloids which were separated by preparative TLC on silica gel plates using petroleum ether : ethylacetate (1:1) as eluent. The faster running band afforded a new alkaloid named "strictimine" (80 mg), $|\alpha|_D = 0^\circ$ which gave an orange coloration with Dragendroff's reagent. The slower running minor band also afforded a new alkaloid, $M^+ = 284$, the structure of which is under investigation.

The major compound gave the molecular ion at m/z = 252. High resolution mass spectrometery on the M^+ ion afforded the exact mass to be 252.2195 which corresponded closely with the mass calculated for the formula $C_{15}H_{28}N_2O$ (252.2201), indicating the presence of three double bond equivalents. The molecular ion peak was confirmed by Fast Atom Bombardment using glycerol as the solubilizing medium. The fragmentation patterns of strictimine was particularly informative, affording the following major peaks in its mass spectrum; ms, m/z (formula, %); 252.2195 (M^+, $C_{15}H_{28}N_2O$, 17%); 223.1805 ($C_{13}H_{23}N_2O$, M^+-Et, 6%); 140.1080 ($C_8H_{14}NO$, $M^+-C_7H_{14}N$, 22%); 112.1128 ($C_7H_{14}N$, 100%), 83.0861 (C_5H_9N, 8%). The formulae of the ions were established by computer monitored high resolution mass measurements and confirmed by peak matching experiments on important ions. As a result of these studies, the structures of the major fragment ions have been identified. Linked scan measurements were carried out to confirm the fragmentation process given in scheme I. The exact masses of the ions, intensities, formulae and proposed structures are shown in table I.

The mass spectrum indicated that the substance was composed of two ethyl piperidine units. The molecular ion at m/z = 252.2195 lost one ethyl group to afford the peak at m/z = 223.1805. Alternatively it lost one of the ethyl piperidine units to afford the ion at m/z = 140.1080 ($C_8H_{14}NO$) which contained the remaining ethyl piperidine unit and a carbonyl group. The base peak at m/z = 112.1128 for the ethyl piperidine moiety was shown by linked scan measurements to arise from the ion at m/z = 140.1080. The facile loss of C=O from the fragment at m/z = 140.1080 ($C_8H_{14}NO$) to afford the ions at 112.1128 ($C_7H_{14}N$) indicated that the carbonyl group was not a part of the piperidine ring but was bonded externally to one of the ring carbon atoms or to the nitrogen. Furthermore the separate losses of the two ethyl groups from fragment (I) to (II) and from fragment (IV) to (V), as demonstrated by linked scan and high resolution experiments, further supported the presence of two different ethyl piperidine containing moieties in the alkaloid. Attempted reduction with sodium borohydride failed to afford the corresponding alcohol indicating that the carbonyl group was not present as a ketone.

The substance showed the lack of any chromorophoric grouping in its UV spectrum. The IR spectrum showed the presence of a carbonyl group; IR, $\nu_{max}(CHCl_3)$ cm^{-1}, 3650, 3400 (C-H stretching), 2850 (C-H), 1710 (C=O), 1615, 1450, 1370, 1140, 1100, 970, 930, 850 and 600.

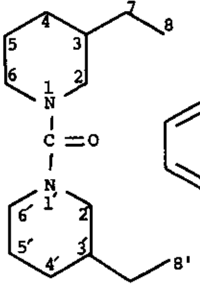
The proton NMR spectrum ($CDCl_3$) showed the presence of a 6-H distorted triplet centred at δ 0.89 (J=6.5Hz) which was assigned to the methyl protons of the two ethyl groups. Two downfield multiplets centred at δ 3.52 and δ 3.65 were assigned to the C-2 α and β protons. Spin-spin decoupling indicated that the C-3 and C-3' methine protons were located as multiplets at δ 2.35. The C-6 methylene protons resonated as multiplets in the region of δ 2.51- δ 2.8. The other methylene protons afforded complex multiplets in the region of δ 1.5- δ 2.1.

The C-13 NMR ($CDCl_3$) (Table II) of strictimine provided strong support for the proposed structure (1). The region between δ 10- δ 55 contained 14 signals and a low field signal was present at δ 165.00. A pair of peaks at δ 52.79 and δ 52.99 were assigned to the C-2 and C-2' carbon atoms adjacent to the nitrogen. The other two low field methylenes resonated at δ 47.53 and δ 47.71 and were assigned to the C-6 and C-6' methylenes α- to the nitrogen. That these signals belonged to methylene carbons and not to methine carbons was confirmed by gated spin echo measurements. This also served to establish that the ethyl groups in the piperidine moieties were not located at the C-2 or C-2' carbon atoms as this would have resulted in the appearance of low field signals for methine carbon atoms. The significant chemical shift difference between C-2 and C-2' on the one hand and C-6 and C-6' on the other indicated that the ethyl groups were not located at C-4 or C-4' which would have resulted in the disappearance of any major chemical shift differences between C-2/C-2' and C-6/C-6', and therefore left only C-3 and C-3' for the location of the ethyl groups. This would reasonably explain the chemical shift differences between C-2/C-2' and C-6/C-6' due to the unsymmetrical substitution of the molecule.

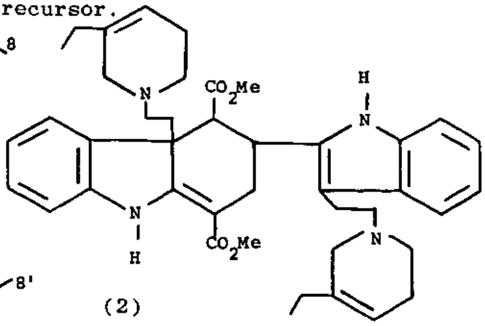
Two peaks at δ 37.67 and δ 37.63 were assigned to the C-3 and C-3' carbon atoms which bear the ethyl groups. It was confirmed by gated spin echo measurements that the carbons responsible for these signals were methine carbon atoms and not methylene carbon atoms.

While the IR spectrum had been deceptive about the nature of the carbonyl group, the presence of a signal at δ 165.00 clearly showed that the carbonyl group was attached to the nitrogen atom. Since gated spin echo measurements had shown that only two CH groups were present(which were assigned to the carbon atoms bearing the ethyl groups)the attachment of the carbonyl group to the ring carbon atoms could be ruled out as it would have generated additional low field methine groups. On the basis of the above spectral data, strictimine is assigned structure (1).

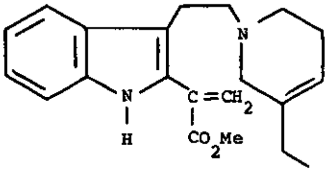
It is interesting that no simple alkaloids bearing an ethyl group at the 3-position of a piperidine ring are known. It is also noteworthy that presecamines (2),which are formed by the dimerisation of secodine molecules (3),have been isolated from *Rhazya stricta*. Strictimine could arise in the plant by a fragmentation of presecamine or secodine systems to afford the 3-ethyl piperidine units which could then combine with an appropriate electrophilic carbonyl containing precursor.



(1)



(2)



(3)

TABLE-I: m/z, formulae, intensities and structures of ions.

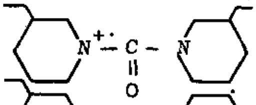
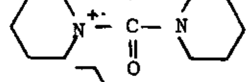
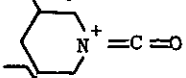
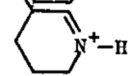
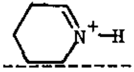
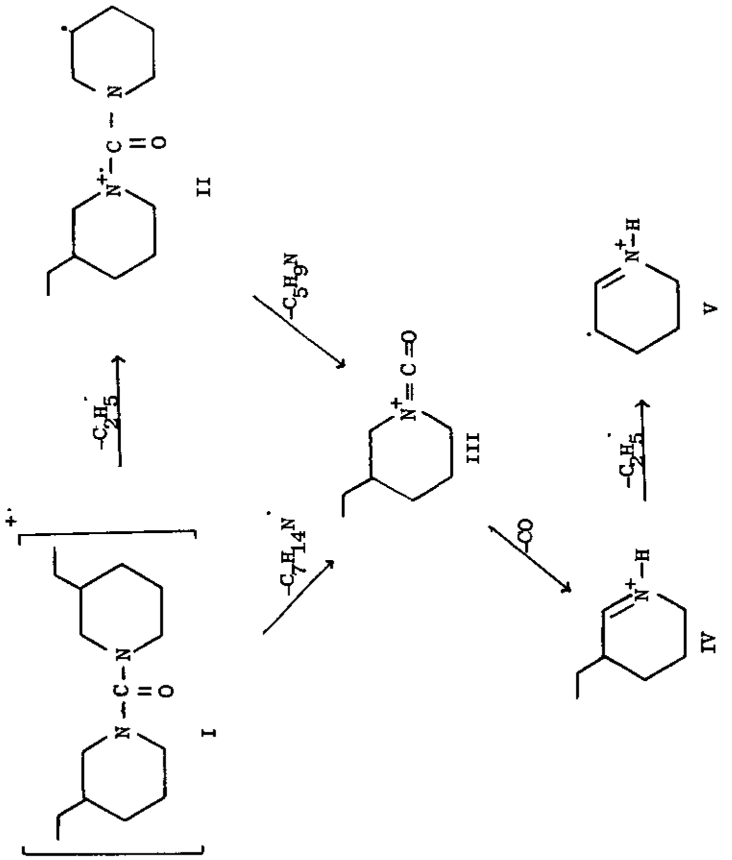
IONS	m/z	FORMULAE	INTENSITIES	PROPOSED STRUCTURES
I	252.2195	$C_{15}H_{28}N_2O$	17	
II	223.1805	$C_{13}H_{23}N_2O$	6	
III	140.1080	$C_8H_{14}NO$	22	
IV	112.1128	$C_7H_{14}NO$	100	
V	83.0861	C_5H_9N	8	

TABLE-II: ^{13}C NMR of Strictimine

CARBON NOS.	CHEMICAL SHIFT δ	NATURE OF CARBON
2	52.79	$N-CH_2$
2'	52.99	$N-CH_2$
3	37.67	CH
3'	37.63	CH
4	29.57	CH_2
4'	31.15	CH_2
5	25.26	CH_2
5'	26.71	CH_2
6	47.53	$N-CH_2$
6'	47.71	$N-CH_2$
7	29.22	CH_2
7'	29.04	CH_2
8	11.31	CH_3
8'	13.99	CH_3
9	165.00	>C=O

REFERENCES

1. R.N.Chopra, S.L.Nayar and I.C.Chopra, "Glossary of Indian Medicinal Plants", C.S.I.R., New Delhi, 212 (1956).
2. W.Dymock, C.J.H.Warden and D.H.Hooper, "Pharmacographia Indica", Kegan Paul, Trench, Trubner and Company, Ltd., London, Vol.II, 3911 (1893).
3. J.D.Hooper, "Flora of the British India", Reeve and Company, Vol.III, 640 (1875).
4. G.Watt, "Dictionary of Economic Products of India", Part I, W.H.Allen and Co. London, VI, 443 (1982).
5. S.Siddiqui and A.Q.S.Bukhari, *Nature*, 235, 393 (1972).
6. S.Mukhopadhyay, G.A.Handy, S.Funayama and G.A.Cordell, *J.Nat.Prod.*, 44(6), 696 (1981).
7. S.Mukhopadhyay, A.El-Sayed, G.A.Handy and G.A.Cordell, *J.Nat.Prod.*, 46(3), 409 (1983).



SCHEME-I

Received, 26th April, 1984