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Isolation and Structural Elucidation of an Unknown Biologically Active Compound from Myanmar Traditional Indigenous Medicinal Plant *Clerodendrum serratum* SPRENG (Yin-bya-net)

Aye Myint¹, Myint Myint Sein² and Mya Aye³

Abstract

In this Ph.D research work, one well-known Myanmar Indigenous Medicinal Plant, namely *Clerodendrum serratum* Spreng. (Yin-bya-net) was chosen for preliminary phytochemical screening and antibacterial activities. In accordance with phyto test the crude extract of root of this plant responds some chemical constituents, such as, alkaloid, phenolic, lipophilic, polyphenol and glycoside, respectively. Moreover, antibacterial activities of this crude extract in various solvent systems were done by agar well diffusion method on three selected organisms (*Bacillus Subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*). Among them ethyl acetate extract of this plant gave rise to medium activities on all tested organisms. Furthermore a pure white amorphous unknown alkaloid compound (DAM-1) was isolated from ethyl acetate extract of root of Yin-bya-net by some modern separation methods, such as Thin Layer and Column Chromatographic methods. The yield percent of this unknown alkaloid could be calculated as (0.85%) based upon the ethyl acetate crude extract. The molecular formula of this unknown potent alkaloid could be assigned as (C₁₃H₂₁O₄N₃) by some sophisticated spectroscopic methods, such as, FT-IR, ¹H NMR(600 MHz), ¹³C NMR (150MHz), DEPT, HSQC and EI-Mass spectrums.

Introduction

Herbal medicine is the use of herbs for their therapeutic or medicinal value. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbal medicine is the oldest form of health care known to man kind. Many drugs commonly used today are of herbal origin.

In Myanmar, medicinal plants are abundant. Myanmar has a long history of health care system by herbal medicines and medicinal plants as a national heritage. The study of traditional indigenous medicinal plants and their usage in therapy plays a very important role in our country.

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This research has been chemically studied on the interesting up grade level of Myanmar traditional medicines. In the present work , the pure bioactive unknown alkaloid compound, (DAM-1) was isolated from the root of *Clerodendrum serratum* Spreng (Yin-bya-net) by using advanced separation techniques and investigated . Then the complete structure of an unknown bioactive alkaloid compound could be assigned by application of modern instrumental spectroscopic methods .

Botanical Description

| | | |
|----------------|---|-------------------------------------|
| Family | - | Verbenaceae |
| Botanical Name | - | <i>Clerodendrum serratum</i> Spreng |
| Myanmar Name | - | Yin-bya-net |
| English Name | - | Prang- gadawn |



*Clerodendrum serratum*Spreng.



Medicinal Uses

Decoction of roots and leaves are reputed to be useful in the treatment of malaria. The leaves and roots are used externally for tumors and certain skin diseases. Fresh leaf juice is used as an injection into the rectum for ascaris.

Procedure

The roots of *Clerodendum serratum* Spreng. (Yin-bya-net) were collected from Pyinmana Township, Mandalay Division. Then they were cut into small pieces and dried in the shade. The neutral ethyl acetate extract (3.8g) obtained from air dried sample (400g, root of Yin-bya-net) was chromatographed on a silica-gel (70-230 mesh) column, eluting with the solvent system, n-hexane : ethyl acetate as non-polar to polar with various ratio. The white amorphous pure unknown compound (32.4 mg) was obtained and the yield was found to be 0.85% based upon the crude extract.

Antibacterial Test on Pure Compound (DAM-1)

The antibacterial activities of pure compound were rechecked by using agar well diffusion method with three organisms. These results are shown in table (1).

Table (1) Antibacterial Test on Pure Compound

| Sample | Solvent | Organism | | |
|-----------------------|---------|--------------------------|------------------------------|-------------------------------|
| | | <i>Bacillus subtilis</i> | <i>Staphylococcus aureus</i> | <i>Pseudomonas aeruginosa</i> |
| Pure compound (DAM-1) | EtOAc | 15 mm (++) | 17 mm (++) | 18 mm (++) |

Result and Discussion

The FT-IR spectrum Fig(1) shows the characteristic functional groups present in this compound. These groups are described in Table (2).

Table(2) The Functional Groups Present in FT-IR spectrum

| Absorption Band (cm ⁻¹) | Assignments |
|-------------------------------------|---|
| 3417 | O-H stretching vibration of alcohol group |
| 3300 | N-H stretching vibration of amine group |
| 3030 | sp ² C-H stretching vibration of aromatic ring |
| 2931 | Unsymmetrical and symmetrical stretching vibration of saturated hydrocarbon |
| 1666 | C = O stretching vibration of amide group |
| 1589 | C = C ring skeletal stretching vibration of aromatic ring |
| 1458 | C-H in plane bending vibration of allylic hydrocarbon |
| 1373 | C-H out of plane bending vibration of gem-dimethyl group |
| 1118 & 1064 | C-O-C stretching vibration of ether group |
| 756 | C-H out of plane bending vibration of cis- or Z alkene |
| 663 | -NH wagging vibration of amine group |

In accordance with the FT-IR spectrum Fig (1) and DEPT spectrum Fig (2), the kinds of carbons, protons, oxygen and nitrogen are described in the following table.

| Assignments | no. of carbon | no. of proton | no. of oxygen | no. of nitrogen |
|---|---------------|---------------|---------------|-----------------|
| Three sp ² quaternary carbon | 3 | - | - | - |
| Two sp ² methine carbon | 2 | 2 | - | - |
| Four sp ³ methylene carbon | 4 | 8 | - | - |
| Two methoxy carbon | 2 | 6 | 2 | - |
| One sp ³ methyl carbon | 1 | 3 | - | - |
| One carbonyl carbon | 1 | - | 1 | - |
| One - OH | - | 1 | 1 | - |
| One - NH | - | 1 | - | 1 |
| | 13 | 21 | 4 | 1 |

Partial molecular formula is C₁₃H₂₁O₄N, and its partial mass = 255.

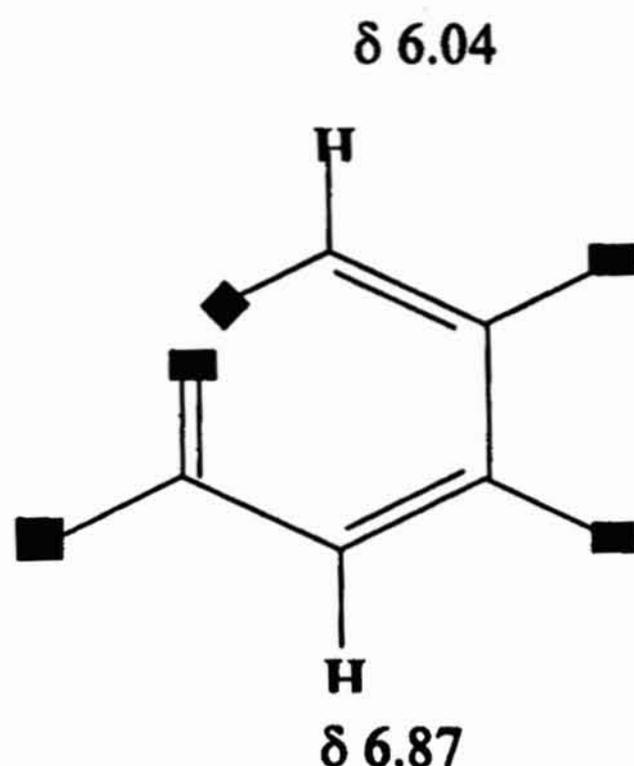
EI mass spectrum shows molecular mass = 283

Thus the remaining mass = 28 (2 N atoms).

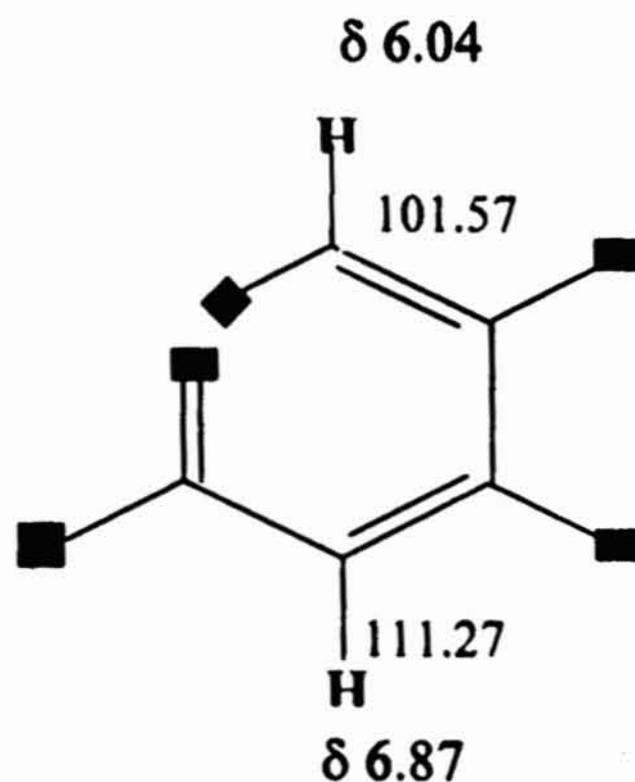
∴ the real molecular formula must be C₁₃H₂₁O₄N₃.

Structure Elucidation of Pure Unknown Compound (DAM-1)

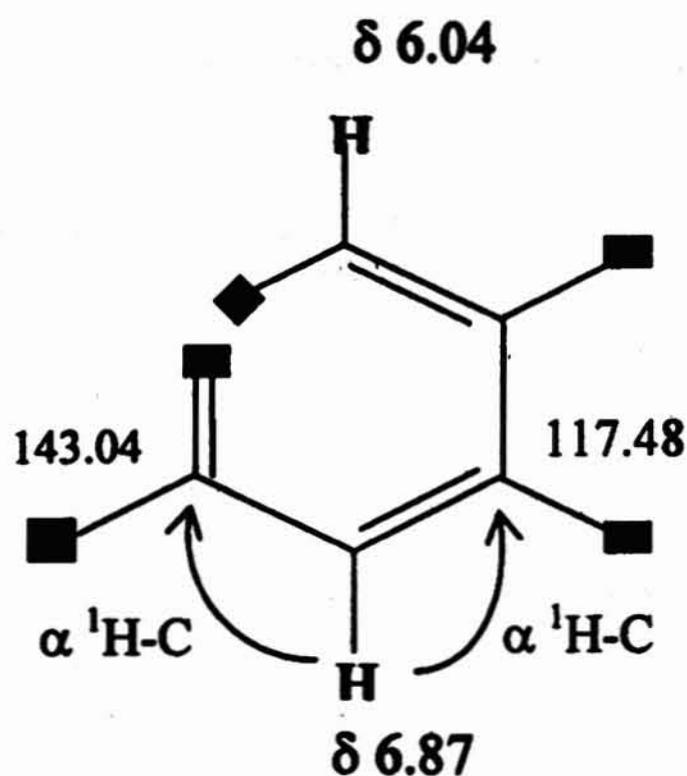
In the ^1H NMR spectrum, the appearance of two singlet protons (δ 6.04, δ 6.87 ppm) indicates the para oriented aromatic fragment.



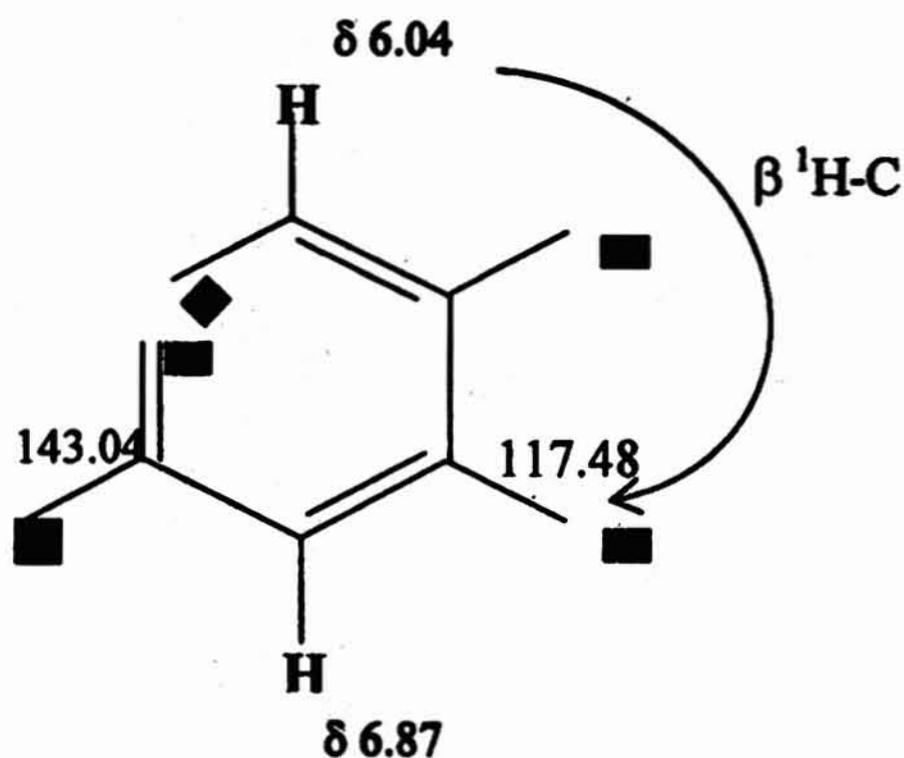
Moreover, HSQC spectrum displays ^1H -C direct coupling as indicated below.



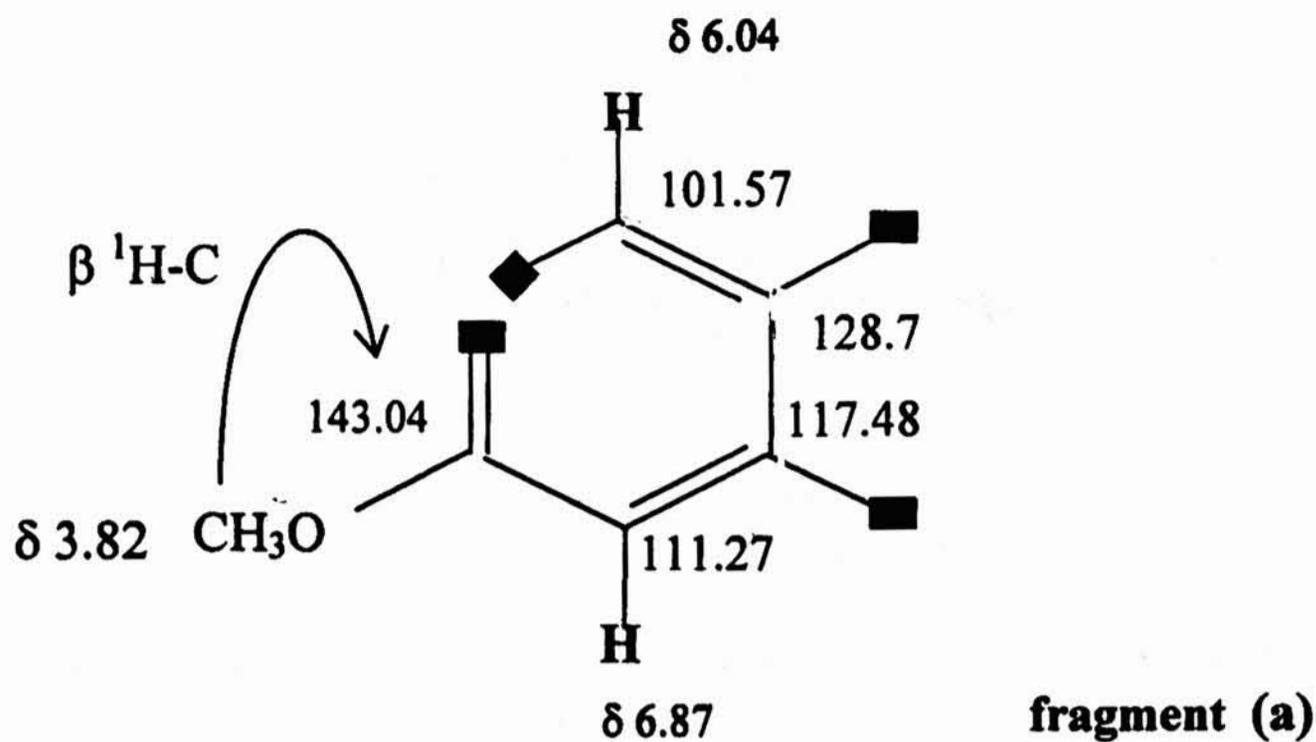
On the other hand, the occurrence of α - ^1H -C long range signal of the aromatic proton (δ 6.87 ppm) with both of sp^2 quaternary carbon (δ 143.04, δ 117.48 ppm) in HMBC spectrum implies the following extended fragment.



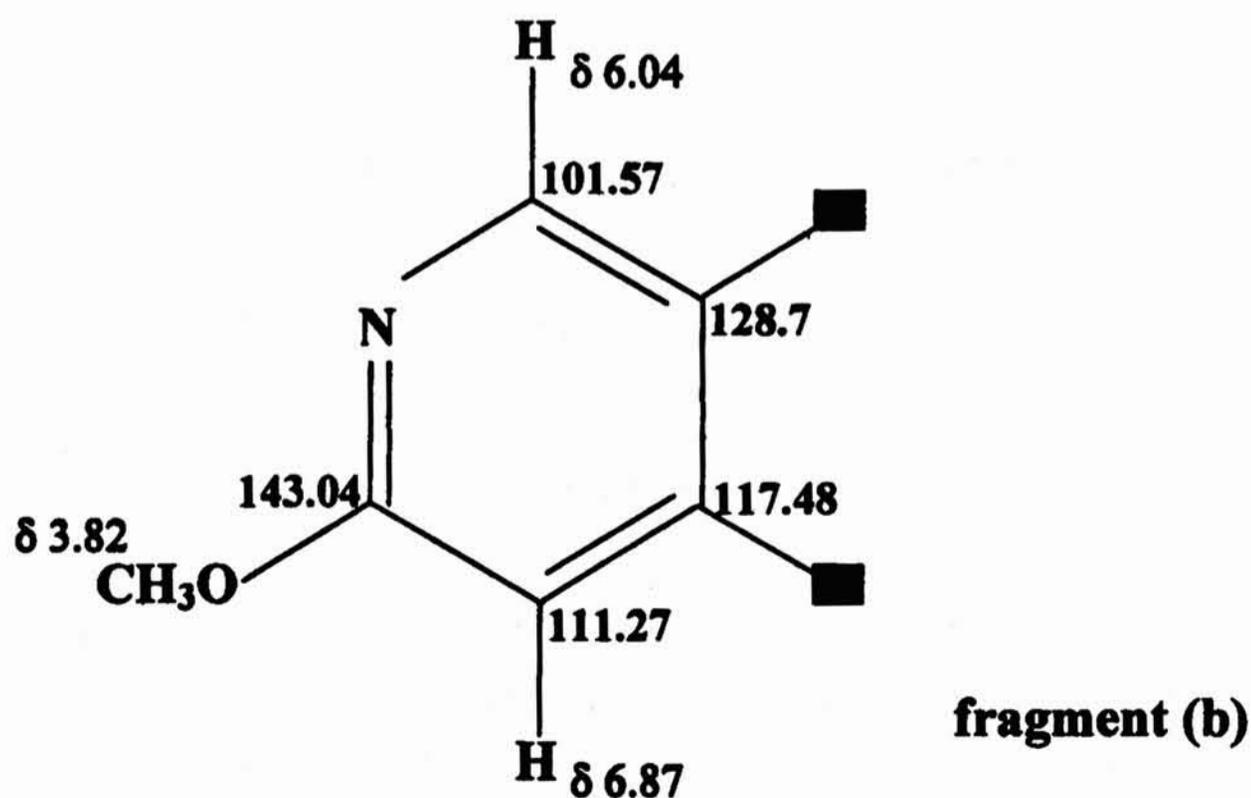
In addition, this spectrum also represents β - $^1\text{H-C}$ long range signal of the aromatic proton (δ 6.04 ppm) with sp^2 quaternary carbon (δ 117.48 ppm) which gave rise to the following fragment.



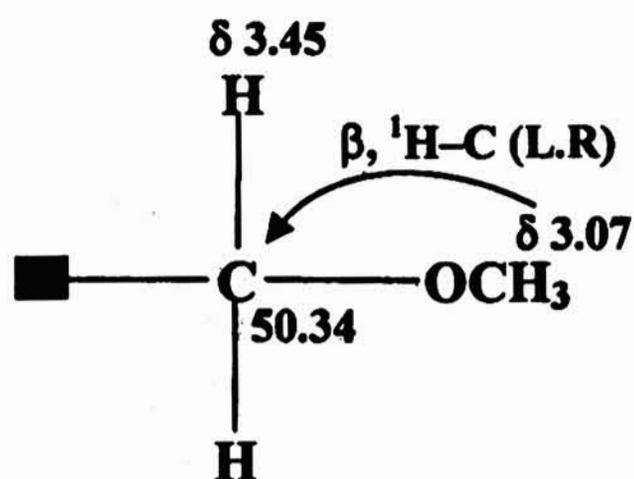
The observation of fragment (a) could be done by HMBC spectrum in which the methoxy signals (δ 3.82 ppm) responds β $^1\text{H-C}$ long range coupling with sp^2 quaternary carbon (δ 143.04 ppm) as indicated below.



In fragment (a), only five sp^2 carbons (δ 143.04, δ 128.7, δ 117.48, δ 111.27 and δ 101.57 ppm) could be detected except carbonyl carbon (δ 167.98 ppm). To complete the three unsaturated groups, the remaining element should be one hetero atom, such as N, O or S. Among them, the most reliable hetero atom should be trivalent one nitrogen and hence, the following pyridine derivative fragment (b) could be assigned.

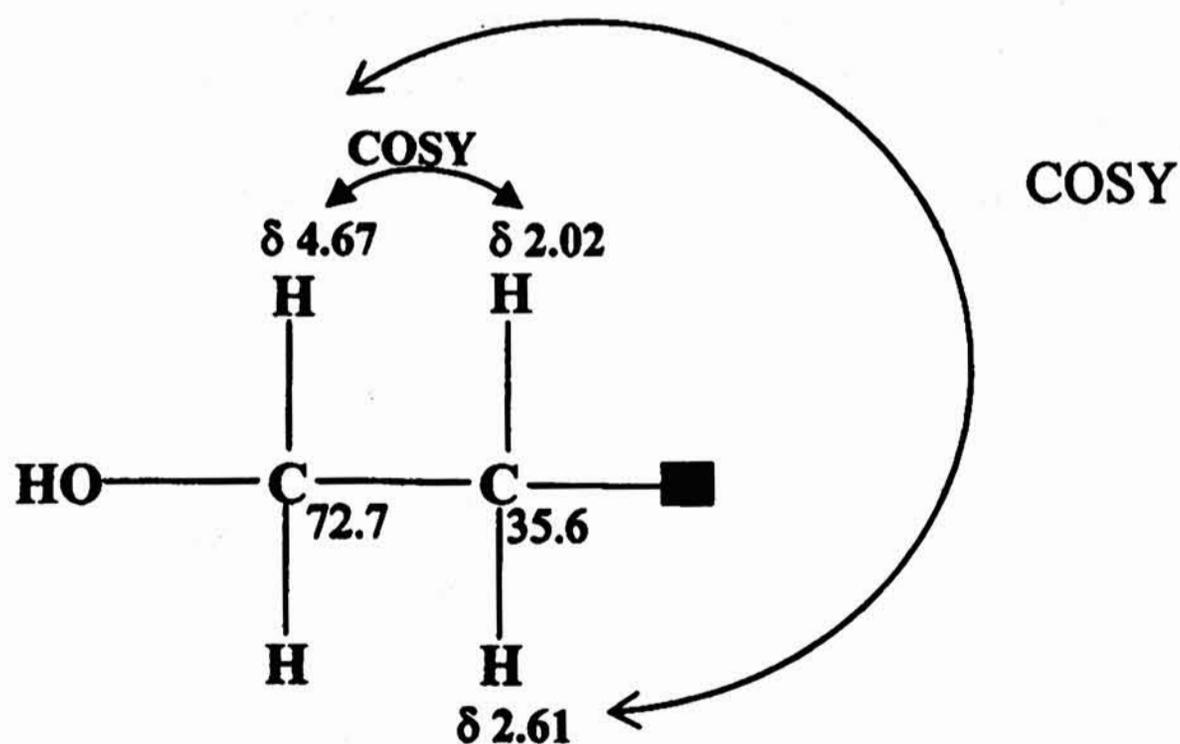


Furthermore, the following fragment (c) could be determined by HMBC spectrum in which β - $^1\text{H-C}$ long range signal between methoxy protons (δ 3.07 ppm) and sp^3 methylene carbon (δ 50.34 ppm) is observed.



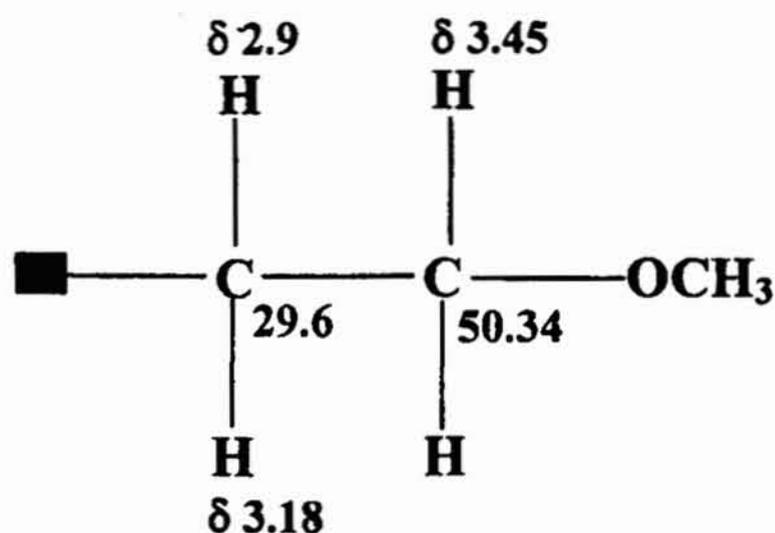
fragment (c)

On the other hand , the carbinol protons ($\delta 4.67$ ppm) and another methylene protons ($\delta 2.02$ ppm and $\delta 2.61$ ppm) are also coupled each other with medium square matrix area in COSY spectrum which leads to the following fragment (d).



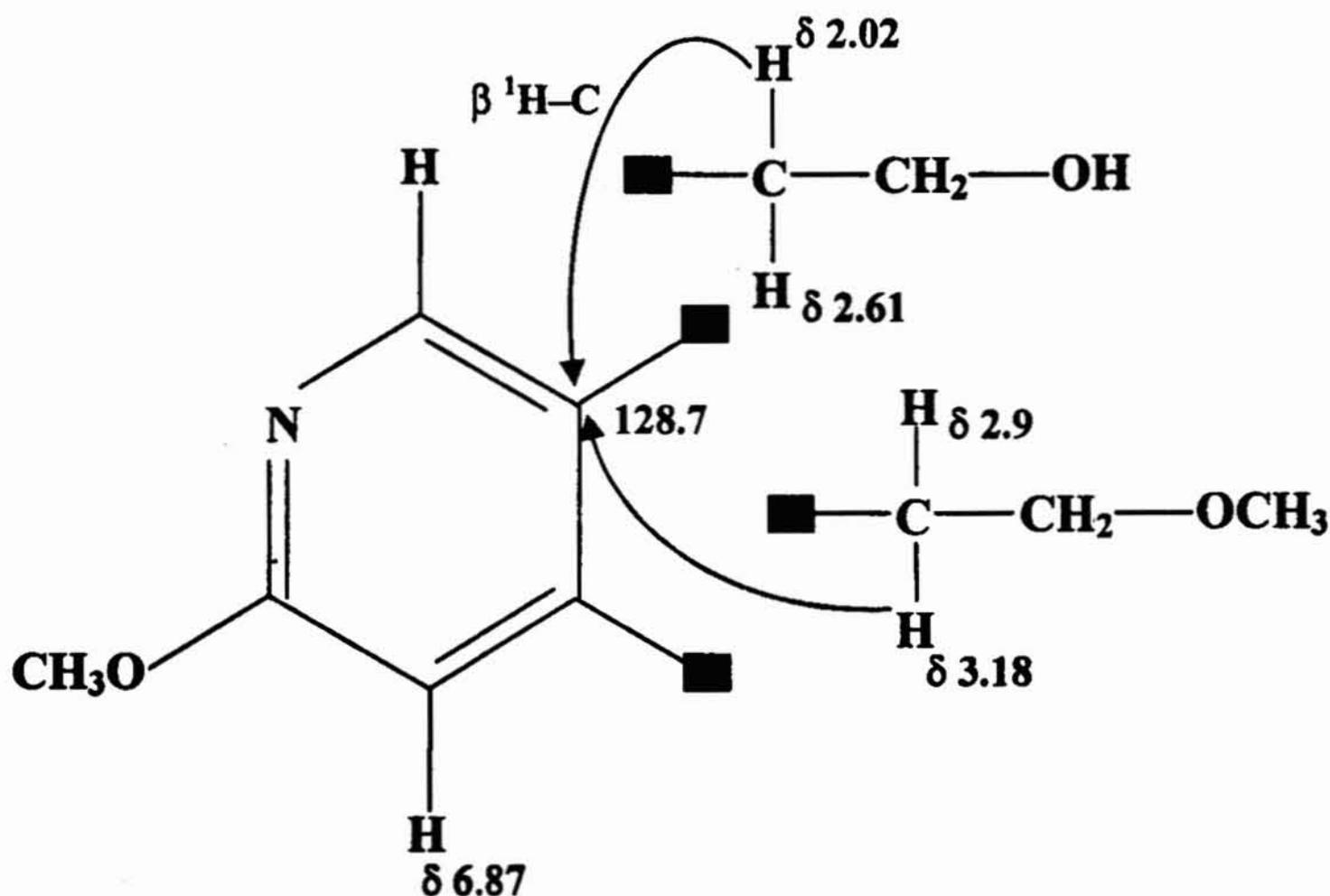
fragment (d)

In DQF-COSY spectrum, the determination of medium graphic area between methylene protons ($\delta 3.45$ ppm) and another methylene protons ($\delta 3.18$, $\delta 2.9$ ppm) displays the following fragment (e) .

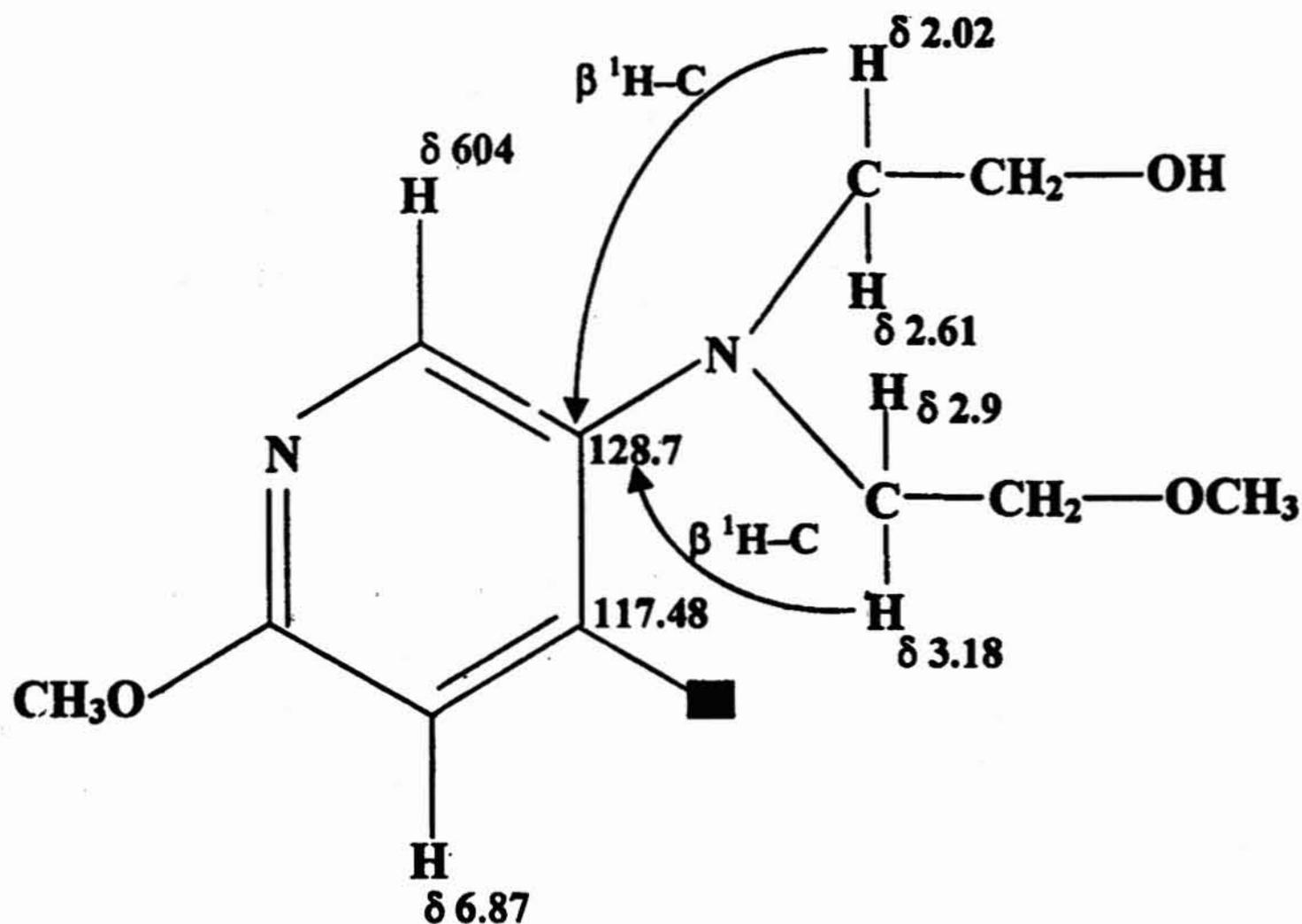


fragment (e)

On the other hand, in HMBC spectrum the existence of β $^1\text{H-C}$ long range signals of both of methylene groups (δ 2.02 ppm, δ 2.61 ppm) and (δ 2.9 ppm, δ 3.18 ppm) with sp^2 quaternary carbon (δ 128.7 ppm) leads to the following tentative fragment.

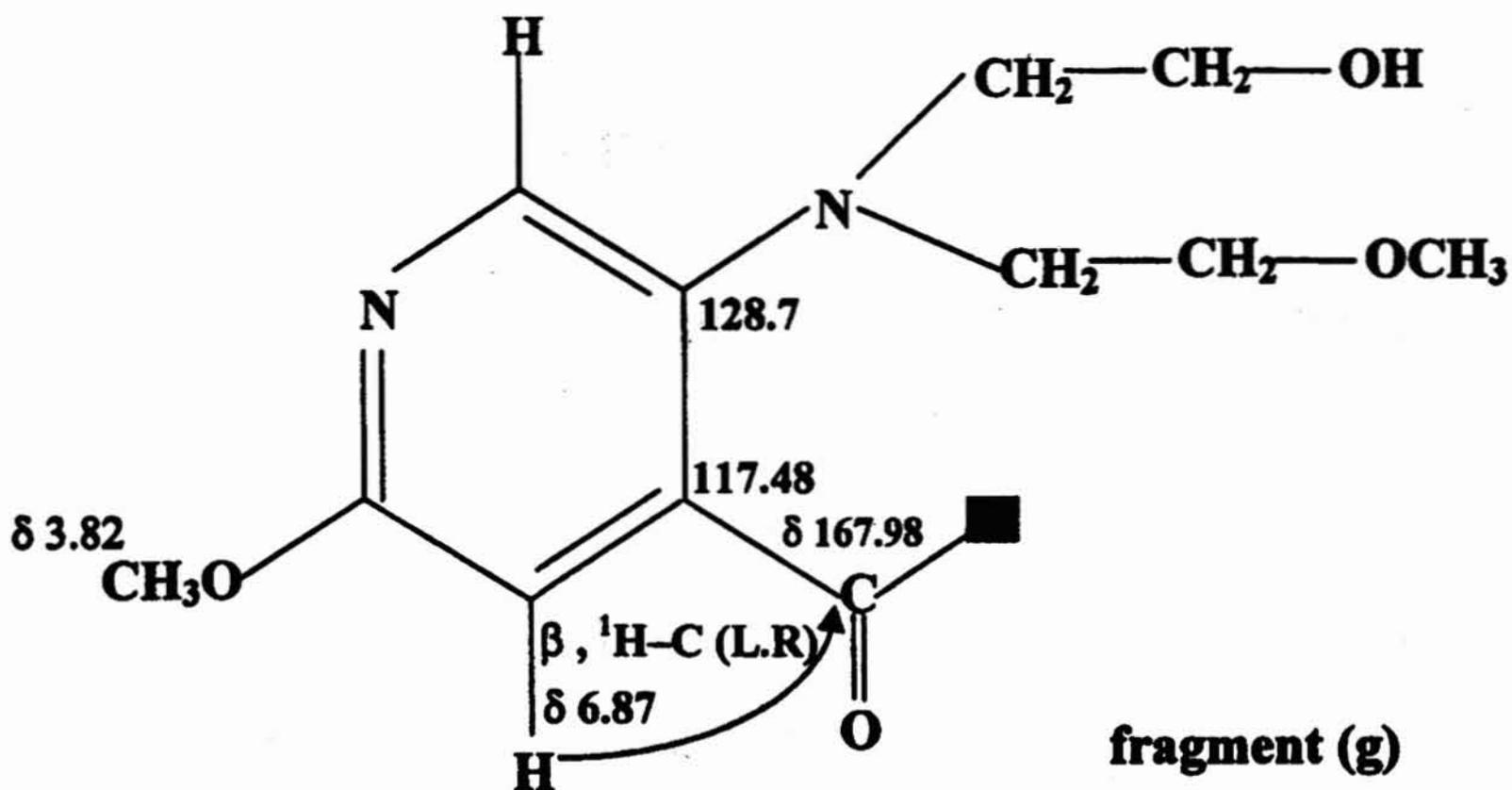


But the appearance of down field chemical shift of the above two methylene groups should be attached to the nitrogen atom giving rise to the following fragment (f).



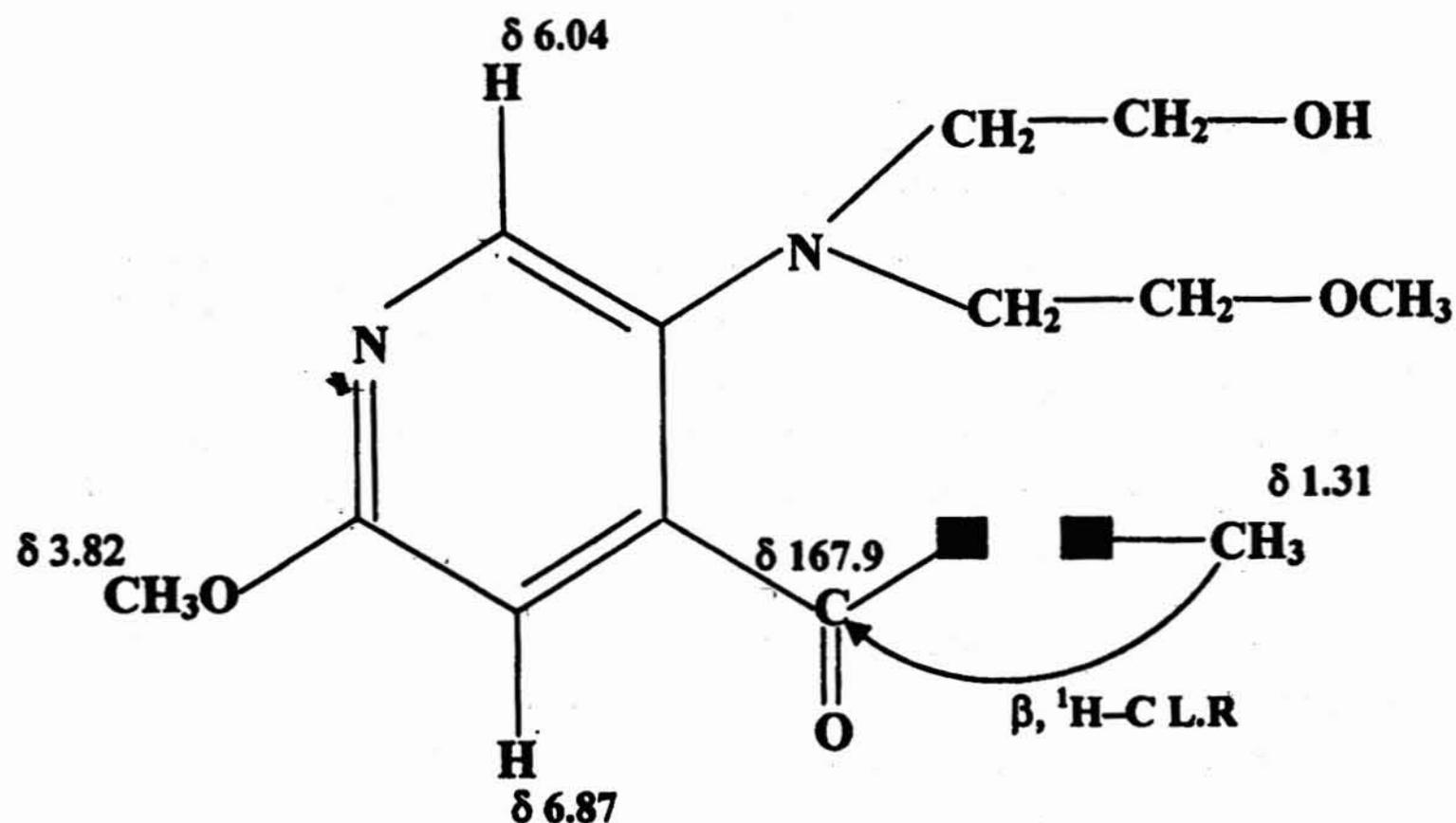
fragment (f)

Furthermore, β $^1\text{H-C}$ long range signal between the aromatic proton (δ 6.87 ppm) with carbonyl carbon (δ 167.98 ppm) which implies more longer fragment (g) is observed.



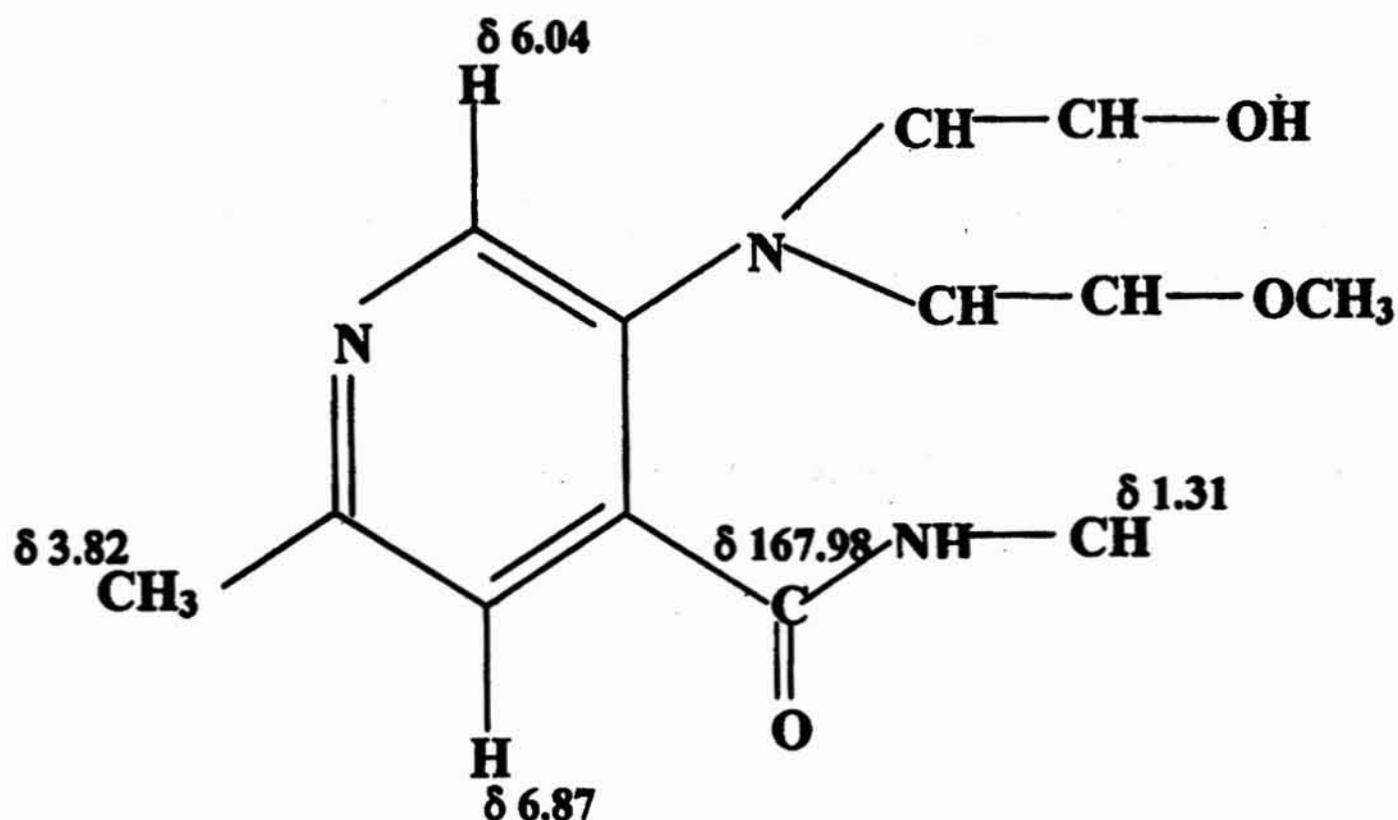
fragment (g)

The HMBC spectrum also displays β $^1\text{H-C}$ long range signal between singlet methyl group (δ 1.31 ppm) and carbonyl carbon (δ 167.98 ppm) leading to the following estimated fragment (h).



fragment (h)

The partial molecular formula of the above fragment could be calculated as $\text{C}_{13}\text{H}_{20}\text{O}_4\text{N}_2$ and hence the remaining unassigned fragment is $(\text{C}_{13}\text{H}_{21}\text{O}_4\text{N}_3 - \text{C}_{13}\text{H}_{20}\text{O}_4\text{N}_2) = \text{NH}$. Logical correlation of this (NH) group with carbonyl carbon and the singlet methyl group (δ 1.31 ppm) can accomplish the complete structure elucidation of this unknown alkaloid.



Complete structure of unknown compound (DAM-1)

I.U.P.A.C name of this compound determines as 5-[(2-hydroxy ethyl)-(2-methoxy ethyl)-amino]-2-methoxy-N-methyl isonicotinamide.

Conclusion

In this research work, one of Myanmar indigenous medicinal plants (Yin-bya-net) was selected to analyse chemically. An unknown alkaloid compound could be isolated as a white amorphous from the root of (Yin – bya -net) applying advanced chromatographic methods , such as Thin Layer and Column Chromatograms. The yield percent was calculated as(0.85%) based upon the ethyl acetate crude extract. Furthermore the antibacterial activities of this pure compound was checked by using agar well diffusion method with three organisms.

Meanwhile, the molecular formula of this unknown alkaloid could be determined as ($C_{13}H_{21}O_4N_3$) by applying some advanced spectroscopic techniques, such as , FT-IR , 1H NMR , ^{13}C NMR , DEPT , HSQC and EI-Mass spectral data , respectively . The existence of odd number (3) nitrogen atoms in this formula with the odd number of molecular mass (283) agrees with the "Nitrogen Rule " .

From the overall assessment of the present investigation, it may be informed that the structure of unknown bioactive compound isolated from the root of Myanmar medicinal plant, *Clenodndrum serratum* Spreng: (Yin-bya-net) can be elucidated .

Since the above pure isolated compound have medium antibacterial action against the three microorganisms: namely; *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* by agar well diffusion method, the above compound may be utilized in the traditional medicine formulation as the mild antibacterial agent.

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I would like to express my deepest gratitude to my supervisors, Dr. Mya Aye, Rector, University of Mandalay and Dr. Myint Myint Sein, Professor and Head , Department of Chemistry, University of Mandalay, for their supervision, helpful advice, numerous invaluable suggestions and kind guidance throughout the course of this research work.

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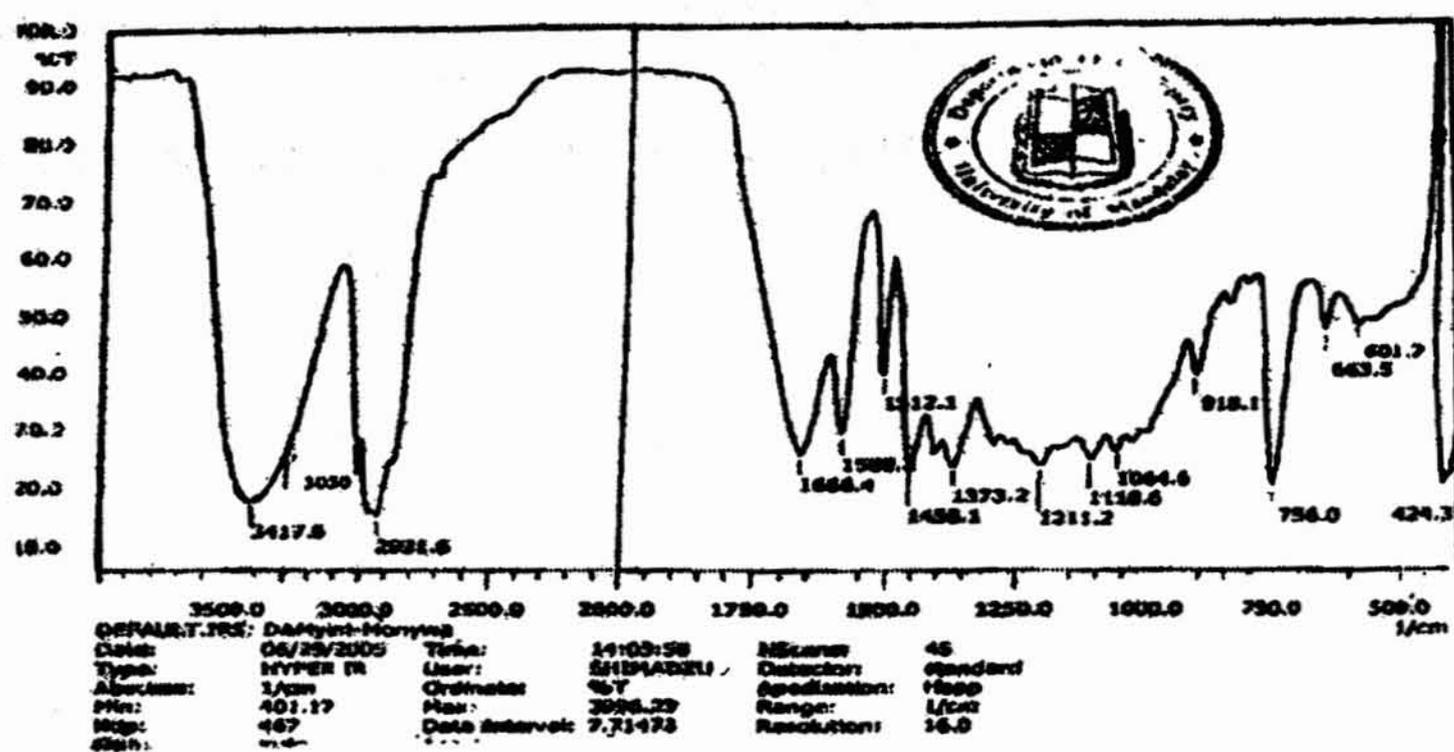
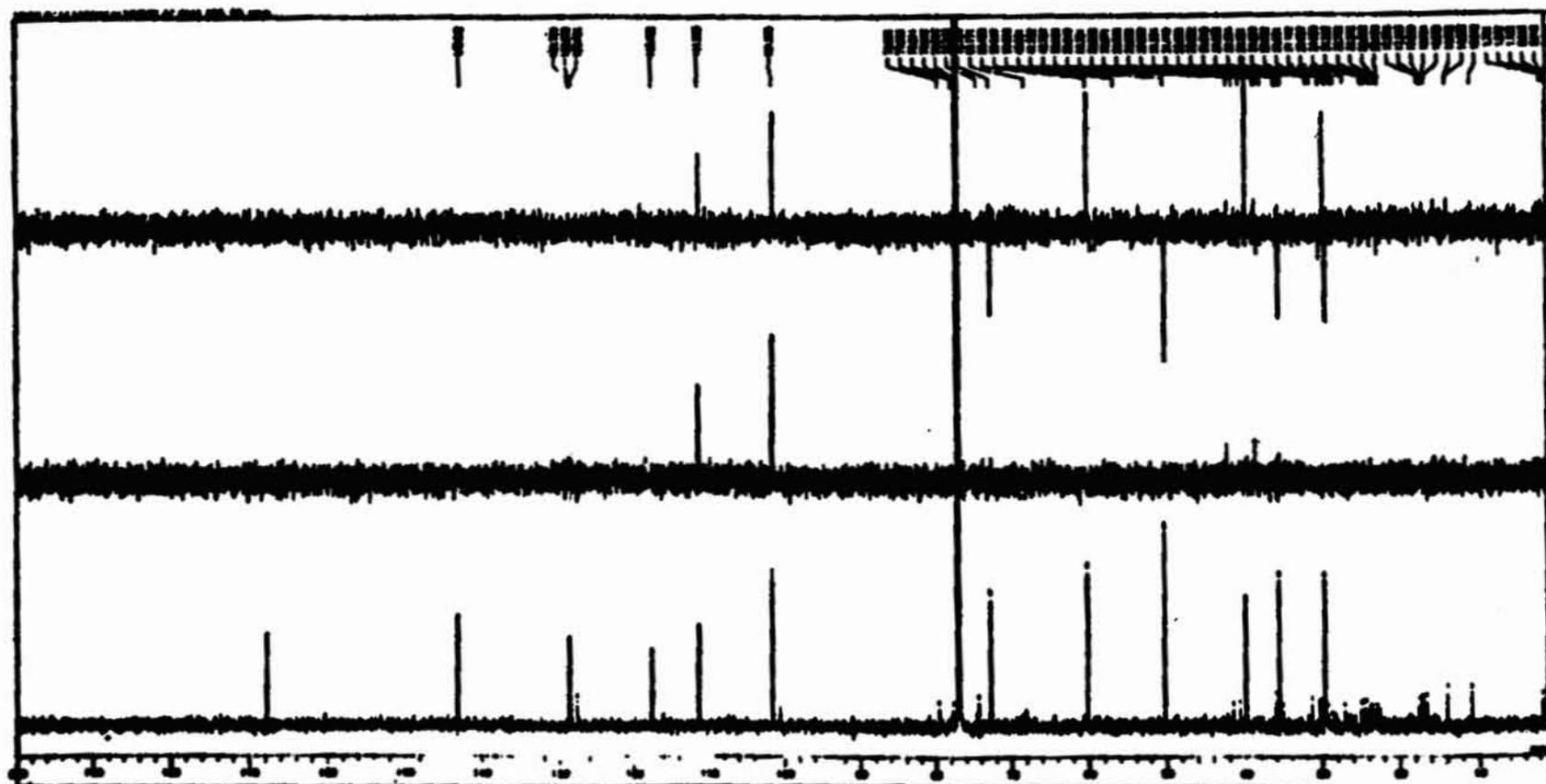


Fig (1). FT-IR spectrum of unknown compound (DAM-1)



Fig(2). DEPT spectrum of unknown compound (DAM-1)

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