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# Isolation and Characterization of Alkaloids Extracted from Medicinal Plant in Malaysia: *Alstonia macrophylla*

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## Abstract

The purpose of this study was to isolate and characterize alkaloids from the leaves of Alstonia macrophylla collected from Penang Island, Malaysia. Various chromatography methods, namely thin layer chromatography, column chromatography and centrifugal chromatography were used to isolate and purified extract. Identification of the isolated pure alkaloids was based on their spectral data and various spectroscopic methods. A total of 19 alkaloids were isolated, 15 alkaloids were characterized; the remaining four alkaloids were suspected as new derivative and subjected to be further elucidated. Eight out of 15 alkaloids isolated were varied with alkaloids reported in East Coast, Malaysia studies.

## Introduction

*Alstonia macrophylla is* belongs the genus *Alstonia;* tribe Plumerieae, subfamily Plumeriodeae and family of Apocynaceae. This genus consists of about 40 species distributed over the tropical parts of Central America, Africa and Asia, with the center of diversity in the Melesia region [1-3]. A total of 19 species of *Alstonia* are found in Melesia, which includes Malaysia, Singapore, Indonesia, Brunei, Philipines, and Papa New Guinea [1]. Of these, eight are found in Malaysia, including *A. macrophylla*.

This plant is commonly known as Hard alsonia, Hard milkwood or Big-leaved macrophyllum [4,5]. In Malaysia, it is termed as '*Pulai penipu bukit*' in Penisular; '*Pulai daun besar*' or '*Sayongan*' in Sabah [4]. *A. macrophylla* is also locally recognized as '*Pule Batu*' in Indonesia; '*Batino*' in Philippines; '*Tung Fa*' in Thailand or '*Chuharoi*' in India.

It is an evergreen green tree measuring about 15-25 meters in height and one meter in diameter. The trunk and branches contain white latex. The bark is smooth and light greyish in colour. The leaf of this plant is generally four verticillate, ovate to elliptic and the upper surface is dark green and glaucous while the lower surface is in lighter green. The fruits are about 30 cm long, green in colour and filled with many small hairy seeds that are dispersed far and wide by the wind. The heartwood is yellowish, with a straight and shallowly interlocked grain with a moderately fine to rather coarse texture [1,6].

This plant has been reported extensively used in traditional medicines for the treatment of various ailments. In India, leaves and stem bark of this plant are widely used among the tribal populations of the Bay Islands to treat stomach ache, skin diseases and urinary infections [7]. In Thailand, it is a general tonic, aphrodisiac, anti-choleric, anti-dysenteric, anti-pyretic, emmenagogue, and vulnerary agents. The drink prepared from its leaves and stem bark is used to treat stomach ache and for the putrefaction of urine [5]. The decoction of stem bark has also been reported to have anti-choleric and vulnerary effects. In the Philippines, the bark is used to treat fatigue, diabetes, and to expel worms from the intestines [6].

Literature review of the biological and pharmacological studies of this plant showed that different plant parts, different location of collection and extraction solvent had demonstrated different significant biological activities. For instance, the methanol extract of leaves has shown anti-oxidant, anti-diabetic, anti-inflammatory, anti-pyretic, anti-fertility and anti-protozoal octets [4]. While, the root bark extract found to possess anti-malarial activity. However, latest study revealed that the leaf extract of *A. macrophylla* showed minimum antibacterial and antimalarial activity [8].

Extraction is a crucial step in screening the biological and pharmacological activities of plant extract, while, isolation is a mandatory step of determining and characterizing pure alkaloids. As the target pure alkaloids vary in polarity and thermal stability, a combination of conventional chromatography methods (Thin layer chromatography, column chromatography, etc.) and instrumentation technologies (CTLC, HPLC, LC/MS, etc.) are commonly used to ensure efficient isolation process [9,10].

The aim of the present research is to present pure alkaloids isolated and characterized from *A. macrophylla* collected from Penang Island, Malaysia; to compare the variability of alkaloids collected from other parts in in Malaysia.

## Materials and Methods

### Plant Collection

The leaves of *Alstonia macrophylla* (labelled as PG9L) were collected from Penang Island, Malaysia.

### Extraction

The leaves of *Alstonia macrophylla* were soaked in 95% distilled ethanol for several days at room temperature. The ethanol extract was decanted and concentrated using a rotary-evaporator. The concentrated ethanolic extract was added slowly, into 3% w/v tartaric acid with stirring. The solution was then filtered through kieselguhr to remove the non-alkaloid substances which are precipitated. The residue was washed with 3% w/v tartaric acid a few times and the washings were combined with the bulk filtrate. The filtrate was then basified with concentrated ammonia to around pH 9-10 with cooling, and the liberated alkaloids were then extracted exhaustively with chloroform. The extracted procedure was repeated three times to ensure that all the alkaloids were extracted. The combined chloroform extract was washed with distilled water for several times and dried with anhydrous sodium sulphate. The chloroform extract was concentrated by rotary-evaporation.

### Isolation and Purification of Alkaloids

Isolation and purification of the alkaloids were carried out by using various chromatographic methods including thin layer chromatography, column chromatography and centrifugal chromatography. All solvents of analytical grade were distilled prior to use with the exception, of diethyl ether. The solvents used were chloroform, methanol, ethanol, ethyl acetate and hexane. The isolation process was summarised in Figure 1.0

**Thin Layer Chromatography (TLC):** Thin Layer chromatography was used for preliminary detection of alkaloids. It was also for testing and selecting the right solvent system for chromatography. The crude alkaloidal extracts, fractions from column chromatography and centrifuges chromatography and the isolated pure alkaloids were examined by TLC using pre-coated 5cm x 10 cm, aluminium sheet of 0.25 mm thickness. The concentrated alkaloidal solution in chloroform was applied as small spots on the TLC plate by using a small capillary tube. The TLC plate is then placed in the saturated chromatographic tank with the appropriate solvents system for development at room temperature. The developed plate was examined under ultra-violet light (254-365 nm). Active alkaloids will appear as intense dark spots under UV light. Detection of the alkaloids was carried out by spraying the developed TLC plate with Dragendroff's reagent where alkaloids will appear as orange spots.

**Column Chromatography (CC):** Flash chromatography was performed using Merck silica gel 9385 (230-400 Mesh ASTM). The ratio of silica gel to the sample was approximately 30:1 for crude samples and 100:1 for semi-pure fractions. The gel was made into slurry with chloroform before it was packed onto the column and was allowed to equilibrate for at least an hour before use. Chloroform was used as eluent with increasing methanol gradient. Fractions were monitored by TLC and appropriate fractions were combined and where necessary subjected to further separation by re-chromatography or Centrifugal Chromatography.

**Centrifugal Chromatography (CTLC):** Centrifugal chromatography was carried out using a circular plate (chromatotron) measuring 24 cm in diameter with the action of a centrifugal force to accelerate mobile phase flow across the circular plate. The plate is rotated at 80 rpm by an electric motor. The plate was prepared by secured the edge of the plate with cellophane tape to form a mould. Silica gel (7749 Kiesegel 60 $PF_{254}$ gipshaltig, Merck) was added with cold distilled water. The slurry is shaken and is then quickly poured on to the circular glass plate before setting commences. The circular glass plate is rotated while the gel is being poured to obtain an even setting. The plate is then left to air-dry for about an hour before being dried in an oven at 80 °C for about 12 hours. The sample was dissolved in a minimum volume of a suitable solvent and loaded at the centre of the plate while the plate is rotating to form a thin band. Elution is then carried out with the appropriate solvent system. The various fractions are collected, concentrated *in vacuo* and examined by TLC. The solvent systems used were: Chloroform (saturated with ammonia); Diethyl ether (saturated with ammonia); Chloroform and hexane (saturated with ammonia) with increasing with chloroform gradient; Diethyl ether and hexane with increasing diethyl ether gradient; Ethyl acetate and hexane with increasing ethyl acetate gradient; Chloroform and methanol (saturated with ammonia) with increasing methanol gradient.

**Sephadex LH-20 Column Chromatography:** The dried powder of Sephadex LH-20 was allowed to swell in methanol for at least 3 hours before use. The slurry was poured onto the column and allowed to equilibrate to room temperature. The sample was filtered with a 0.45 µm nylon membrane before it was loaded into the column to ensure a longer column life. The column can be regenerated by washing of the Sephadex LH-20 gel with 2-3 column volumes of eluent, followed by re-equilibration.

## Structure Characterization

$^{1}H$ was obtained by JEOL JNM-LA 400, or JNM-ECA 400 or Bruker Avance III 400 spectrometers. Samples were prepared in $CDCl_3$ with several drops of TMS as internal reference. Spectra of each compound were compared with literature review to determine the compound name. If the spectrum is not available from literature review, the structure will subject to further elucidate by means $^{1}H$ and $^{13}C$ NMR spectra (at 400 and 100 Hz, respectively). Other data, such as mass spectrometry result were obtained for each compound for additional information on structural characterization.

## Results and Discussion

Nineteen pure compounds were isolated. hRf values of the isolated pure alkaloids in various solvent systems were recorded (Table 1). Spectra of each pure compound were compared with the literature. Fifteen pure alkaloids were successfully characterized and labelled from **1** to **15** (Table 2). Amongst 15 alkaloids, only 3 alkaloids (Compound 1, 4 and 5) were isolated as inseparable mixture, others are single pure alkaloids. All compounds obtained were categorized into six groups: Macroline (Compound 1,2,3); Macroline oxindole (Compound 4 and 5); Akuammiline (Compound 6-9); Ajmaline

**Table 1:** $hR_f$ Values of the Isolated Pure Alkaloids in Various Solvent Systems.

| Alkaloid | Weight (mg) | Solvent system | | | | |
|---|---|---|---|---|---|---|
| | | P | Q | R | S | T |
| Compound **1** | 15.1 | 11 | 35 | 20 | 70 | 44 |
| Compound **2** | 83.3 | 11 | 25 | 15 | 68 | 40 |
| Compound **3** | 8.0 | 3 | 10 | 11 | 49 | 26 |
| Compound **4** | 20.3 | 8 | 40 | 25 | 68 | 50 |
| Compound **5** | 64.8 | 8 | 39 | 20 | 70 | 48 |
| Compound **6** | 8.4 | 3 | 4 | 2 | 50 | 9 |
| Compound **7** | 83.4 | 1 | 3 | 2 | 49 | 8 |
| Compound **8** | 149.7 | 1 | 6 | 2 | 44 | 12 |
| Compound**9** | 210.3 | 4 | 9 | 3 | 61 | 15 |
| Compound**10** | 46.7 | 2 | 11 | 5 | 49 | 21 |
| Compound**11** | 312.3 | 6 | 26 | 13 | 61 | 35 |
| Compound**12** | 436.0 | 9 | 30 | 12 | 71 | 38 |
| Compound **13** | 54.2 | 2 | 3 | 2 | 43 | 7 |
| Compound **14** | 98.8 | 1 | 2 | 1 | 38 | 4 |
| Compound **15** | 16.4 | 0 | 0 | 0 | 29 | 0 |

**Remarks:**
P: Chloroform
Q: Ethyl Acetate
R: Diethyl ether
S: Chloroform methanol (1:10)
T: Ethyl Acetate methanol (1:20)

(Compound 10- 12); Corynantheine (Compound 13) and Strychnan (Compound 15). For each group, selected compound's NMR spectra and data were compared and interpreted.

## Macroline group

**Alstophylline (1a) and Alstophyllal (1b):** The 1H NMR spectrum (Table 3, Figure 2) showed overlap involving most of the signals except for a few which were distinguishable. For example, only the H-18 (methyl) and H-21 (aldehyde for 1b, vinylic-H for 1a) signals of both compounds were clearly distinguished, while the H-17 signals were partially overlapped. Other than these, the remaining signals were virtually overlapped. In the case of the 13C NMR data (Table 3), the C-18, C-19, and C-21 signals were clearly distinguishable, while the rest were either overlapped, or occur in pairs, with very similar chemical shifts, which nevertheless, can be distinguished by their relative intensities, since there is a 4-fold predominance of the type-B macroline form. These compounds tend to co-elute during isolation and were frequently obtained as a mixture, with the type-A macroline form predominating. Since the majority of the peaks were overlapped in NMR, the presence of the minor type-A macroline may sometimes escape detection. Compound 1 has been confirmed as Alstophylline (1a) and Alstophyllal (1b) [11].

## Macroline oxindole group

***N*(4)-Demethylalstophylline Oxindole (5a) and *N*(4) Demethylalstophyllal Oxindole (5b):** The 1H NMR spectrum (Figure 3) showed overlap involving most of the signals. The main differences were the presence of a COMe group at C-20 and the

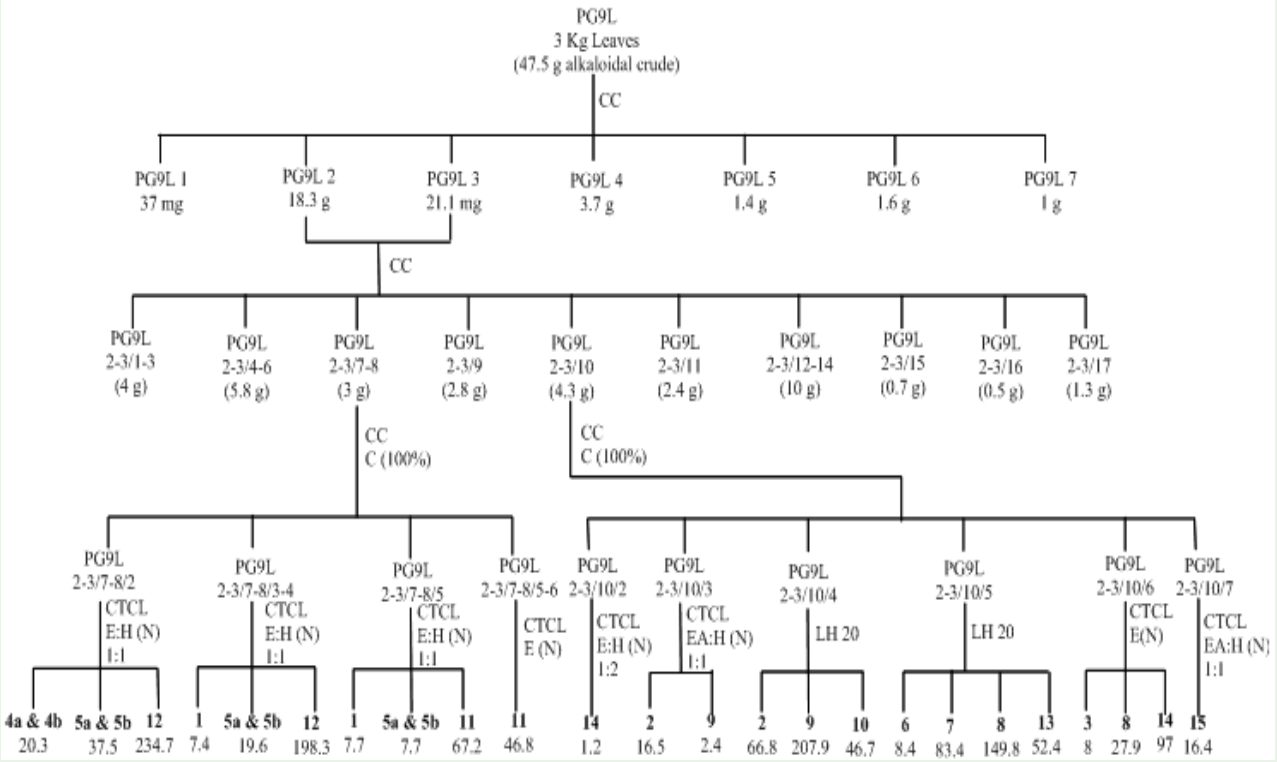



**Figure 1**: Isolation of Alkaloids from the Leaf Extract of *A. macrophylla.*

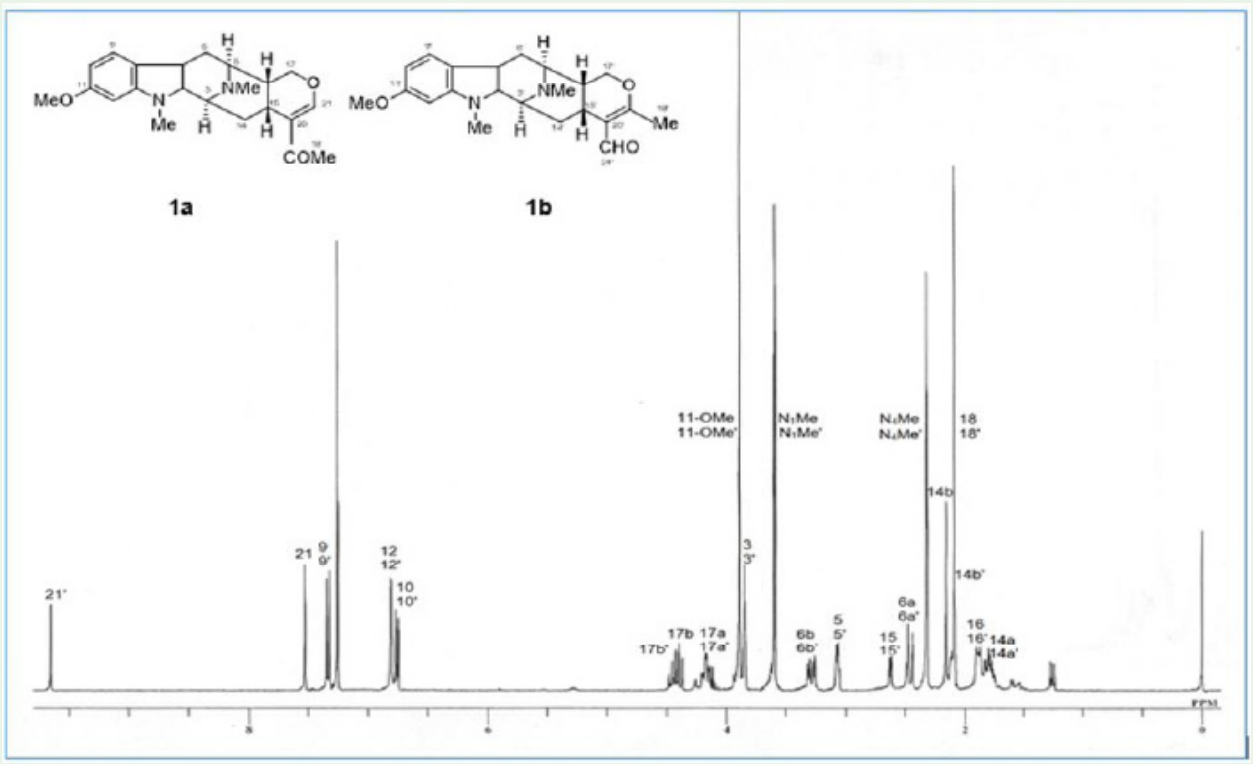



**Figure 2**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of Alstophylline (**1a**) and Alstophyllal (**1b**).

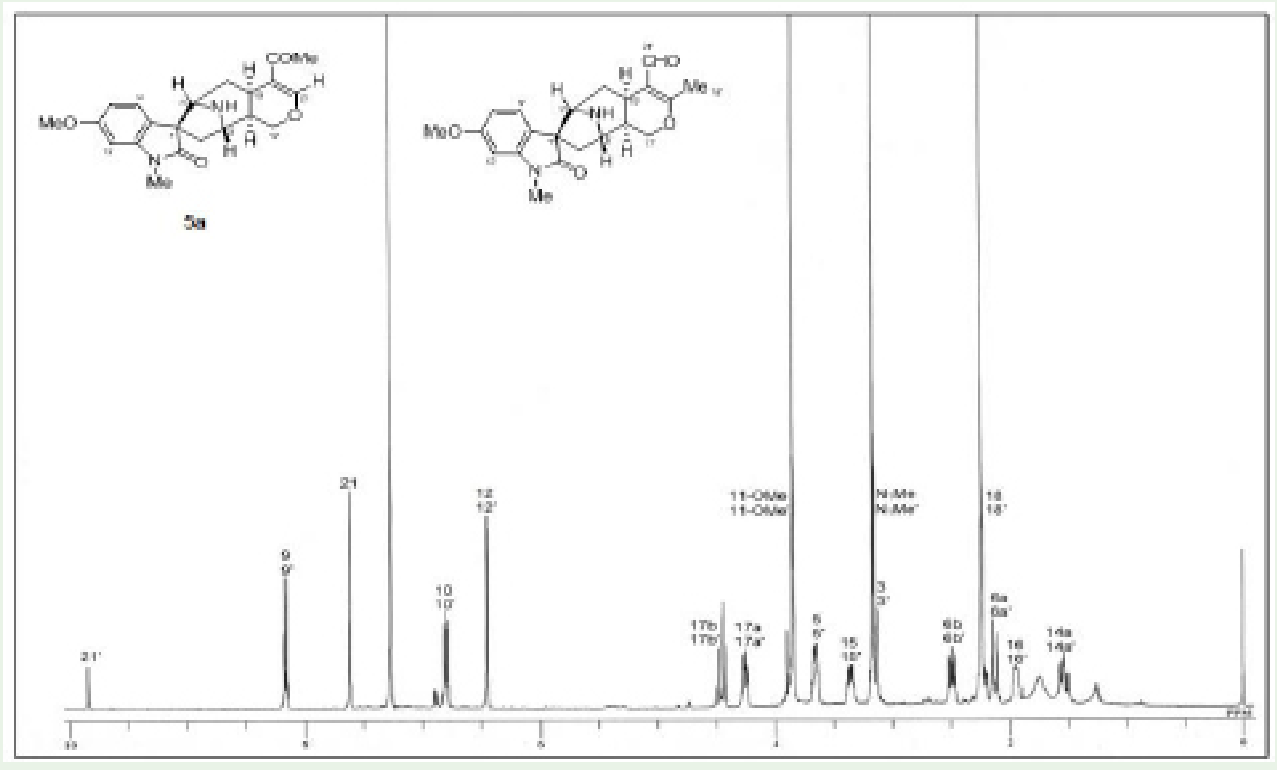
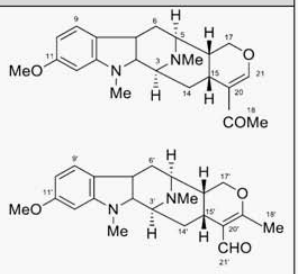
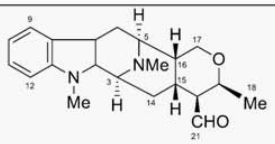
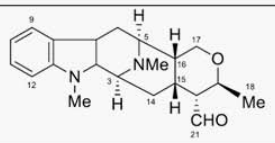
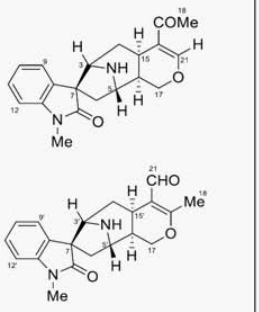
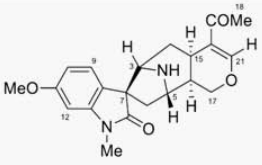
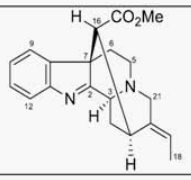
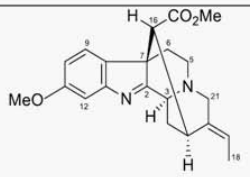
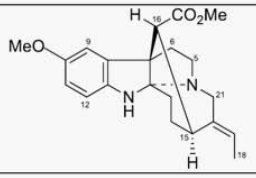
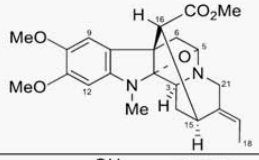
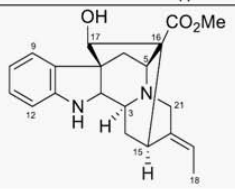
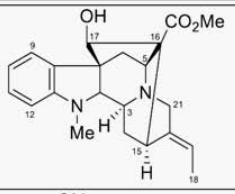
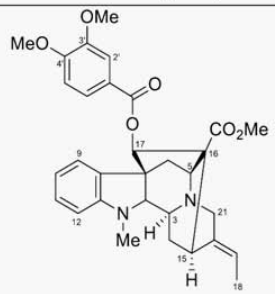
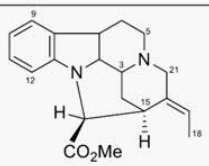
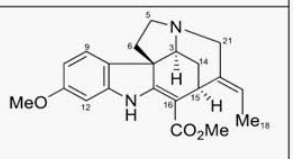
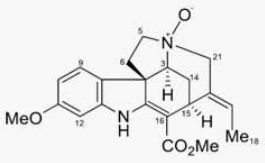



**Figure 3:** $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of *N*(4) Demethylalstophyllineoxindole (**5a**) and *N*(4)-Demethylalstophyllaloxindole (**5b**).

**Table 2:** Summary of Pure Compounds Isolated.

| Comp. | $[\alpha]_D$ | UV spectrum absorption Max. (nm) | ESIMS spectrum $[M + H]^+$ | Formula | Name | Structure |
|---|---|---|---|---|---|---|
| 1 | – 101 (*c*0.04, $CHCl_3$) | 203 (log ε 4.54) 232 (log ε 4.49) 255 (log ε 4.10) 310 (log ε 3.73) | 367 | $C_{22}H_{26}N_2O_3 + H$ | Alstophylline and Alstophyllal [11] |  |
| 2 | +26 (c0.08, $CHCl_3$) | 209 (log ε 3.86) 226 (log ε 4.01) 227 (log ε 2.65) 281(log ε 2.91) 294 (log ε 2.65) | 339 | $C_{21}H_{26}N_2O_2 + H$ | Talcarpine [11] |  |
| 3 | +19 (c0.45, $CHCl_3$) | 205 (log ε 3.95) 228 (log ε 4.21) 280 (log ε 3.00) | 339 | $C_{21}H_{26}N_2O_2 + H$ | *N*(4)-Methyl-*N*(4), 21-*seco*talpinine [11] |  |
| 4 | | | 339 | $C_{20}H_{22}N_2O_3 + H$ | Alstonisine and Alstonal [12] |  |
| 5 | +119 (*c*0.32, $CHCl_3$). | 213(log ε 4.72) 248(log ε 4.39) 296 (log ε 3.97) | 369 | $C_{21}H_{24}N_2O_4 + H$ | N(4)-Demethylalstophylline Oxindole and N(4)-Demethylalstophyllal Oxindole [13] |  |
| 6 | +61 (c0.32, $CHCl_3$) | 219 (log ε 4.07) | 323 | $C_{20}H_{22}N_2O_2 + H$ | Strictamine [14] |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 264 (log ε 3.59)<br>284 (log ε 3.47)<br>296 (log ε 3.35) | | | | |
| 7 | +72<br>(c 0.09, $CHCl_3$) | 214 (log ε 4.15)<br>250 (log ε 3.72)<br>282 (log ε 3.50)<br>299 (log ε 3.04) | 353 | $C_{21}H_{24}N_2O_3$ + H | Methoxystrictamine [14] |  |
| 8 | +162<br>(*c*0.29, $CHCl_3$). | 201 (log ε 4.46)<br>241 (log ε 3.94)<br>316 (log ε 3.52) | 355 | $C_{21}H_{26}N_2O_3$ + H | Nor-vincorine |  |
| 9 | + 15<br>(*c*20, $CHCl_3$) | 205 (log ε 4.62)<br>245(log ε 3.91)<br>303 (log ε 3.71) | 413 | $C_{23}H_{28}N_2O_5$ + H | Quaternine [15] |  |
| 10 | +41<br>(*c*0.63, $CHCl_3$) | 202 (log ε 3.82)<br>245 (log ε 4.19)<br>288 (log ε 3.92) | 353 | $C_{21}H_{24}N_2O_3$ + H | Quebrachidine [15] |  |
| 11 | +58<br>(*c*0.03, $CHCl_3$) | 249 (log ε 3.29)<br>292 (log ε 2.90) | 367 | $C_{22}H_{26}N_2O_3$ + H | Vincamajine [15] |  |
| 12 | +89<br>(*c*1.11, $CHCl_3$) | 204 (log ε 5.50)<br>213 (log ε 5.33)<br>255 (log ε 5.04)<br>294 (log ε 4.81) | 531 | $C_{31}H_{34}N_2O_6$ + H | Vincamajine-17-*O*-veratrate [15] |  |
| 13 | +87<br>(*c*0.08, $CHCl_3$) | 230 (log ε 4.32)<br>284 (log ε 3.85) | 323 | $C_{20}H_{22}N_2O_2$ + H | Pleiocarpamine |  |
| 14 | +39<br>(*c* 0.11, $CHCl_3$). | 209 (log ε 4.05)<br>223 (log ε 4.06)<br>242 (log ε 3.94) | 353 | $C_{21}H_{24}N_2O_3$ + H | 11-Methoxyakuammicine |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 307 (log ε 3.88)<br>327 (log ε 4.01) | | | | |
| 15 | +56<br>(*c* 0.25, $CHCl_3$) | 209 (log ε 4.49)<br>232 (log ε 4.27)<br>253 (log ε 4.16)<br>296 (log ε 3.98)<br>308 (log ε 4.04) | 343 | $C_{21}H_{24}N_2O_4$ + H | 11-methoxyakuammicine-*N*-(4)-oxide |  |

vinyl-H (H-21) in 5a as compared to the presence of the aldehyde function at C-20, together with the vinyl methyl (18-Me) in 5b. The vinyl aldehyde group in 5b was characterized by the signal at $\delta$ 189.4 (C-21) and a 1H singlet at $\delta$ 9.85 (H-21), whilst the carbon resonances at $\delta$ 16.7 and $\delta$ 171 were attributed to C-18 and C-19 respectively. The COMe group in 5a was characterized by the signal at $\delta$ 196.6 (C-19) and $\delta$ 25.0 (C-18). The carbon resonance at $\delta$ 158.0 was attributed to C-21 and a 1H singlet at $\delta$ 7.63 was corresponding to H-21. This characteristic indicating compound 5 is N(4)-Demethylalstophylline Oxindole (5a) and N(4)-Demethylalstophyllal Oxindole (5b) [13].

**Table 3**: $^1$H and $^{13}$C NMR Spectral Data of Alstophylline (**1a**) and Alstophyllal (**1b**)[a].

| | 1a | 1b | | |
|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | - | 131.8 | - | 132.6 |
| 3 | 3.86 m | 53.6 | 3.84 m | 53.9 |
| 5 | 3.09 d (7) | 54.6 | 3.06 d (7) | 54.8 |
| 6a<br>6b | 2.48 d (16)<br>3.30 dd (16,7) | 22.3<br>- | 2.45 d (16)<br>3.29 dd (16,7) | 25.0<br>- |
| 7 | - | 105.8 | - | 105.7 |
| 8 | - | 121.0 | - | 121.0 |
| 9 | 7.34 d (8) | 118.2 | 7.34 d (8) | 118.3 |
| 10 | 6.76 dd (8,1) | 108.1 | 6.76 dd (8,2) | 108.3 |
| 11 | - | 155.9 | - | 155.8 |
| 12 | 6.81 d (1) | 93.2 | 6.81 d 2) | 93.4 |
| 13 | - | 137.2 | - | 137.9 |
| 14a<br>14b | 1.80 td (12,3)<br>2.11 m | 31.8<br>- | 1.77 td (12,4)<br>2.14 m | 32.3<br>- |
| 15 | 2.62 m | 24.9 | 2.61 m | 25.0 |
| 16 | 1.90 m | 38.4 | 1.89 m | 38.5 |
| 17 | 4.18 ddd (11,4, 2)<br>4.42 t (11) | 67.7<br>- | 4.19 ddd (11,4, 2)<br>4.46 t (11) | 67.7<br>- |
| 18 | 2.09 s | 16.5 | 2.17 s | 22.9 |
| 19 | - | 170.0 | - | 195.5 |
| 20 | - | 121.0 | - | 121.0 |
| 21 | 7.53 s | 188.8 | 9.66 s | 157.0 |
| N(1)-Me | 3.60 s | 29 | 3.60 s | 29.1 |
| N(4)-Me | 2.34 s | 41.6 | 2.32 s | 41.7 |
| 11-OMe | 3.89 s | 55.8 | 3.89 s | 56.1 |

[a]$CDCl_3$, 400 and 100 MHz; assignments based on COSY, HMQC and HMBC.

## Akuammiline group

**Strictamine (6) and 11-Methoxystrictamine (7):** The $^1$H NMR spectrum (Table 5, Figure 4) of **6** showed the presence of four aromatic hydrogens ($\delta$ 7.18–7.64) due to an unsubstituted indole chromophore. A singlet signal at $\delta$ 3.73 indicated the presence of a $CO_2$Me group at C-16. The presence of the characteristic ethylidene side chain was indicated by signals observed at $\delta$ 1.57 (H-18) and 5.55 (H-19). The $^{13}$C NMR data (Table 5) of **6** gave a total of 20 carbon resonances. The presence of ethylidene side chain were indicated

**Table 4**: $^1$H and $^{13}$C NMR Spectral Data of *N*(4)-Demethylalstophyllineoxindole (**5a**) and *N*(4)-Demethylalstophyllaloxindole (**5b**) [a].

| Position | 5a | | 5b | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | - | 183.1 | - | 183.1 |
| 3 | 3.16 br s | 63.8 | 3.16 br s | 63.8 |
| 5 | 3.68 m | 56.2 | 3.68 m | 56.2 |
| 6 | 2.14 dd (13.1)<br>2.51 dd (13, 8) | 42.1 | 2.13 dd (13.1)<br>2.50 dd (13, 8) | 42.1 |
| 7 | - | 56.3 | - | 56.3 |
| 8 | - | 121.2 | - | 121.2 |
| 9 | 8.17 d (8) | 126.2 | 8.16 d (8) | 126.2 |
| 10 | 6.81 dd (8, 2) | 106.3 | 6.81 dd (8, 2) | 106.3 |
| 11 | - | 160.0 | - | 160.0 |
| 12 | 6.46 d (2) | 96.9 | 6.46 d (2) | 96.9 |
| 13 | - | 145.3 | - | 145.3 |
| 14 | 1.57 m<br>2.26 m | 31.2<br>- | 1.57 m<br>2.26 m | 31.2<br>- |
| 15 | 3.35 m | 24.2 | 3.35 m | 24.1 |
| 16 | 1.95 m | 37.0 | 1.95 m | 37.0 |
| 17 | 4.26 ddd (11,4,2)<br>4.47 t (11) | 68.5 | 4.28 ddd (11,4,2)<br>4.53 t (11) | 68.6 |
| 18 | 2.24 s | 25.0 | 2.25 s | 16.6 |
| 19 | - | 196.6 | - | 171.0 |
| 20 | - | 122.0 | - | 118.4 |
| 21 | 7.63 s | 158.0 | 7.83 s | 189.4 |
| *N*(1)-Me | 3.17 s | 26.3 | 3.17 s | 26.3 |
| 11-OMe | 3.85 s | 55.5 | 3.86 s | 55.5 |

[a]$CDCl_3$, 400 and 100 MHz; assignments based on COSY, HMQC and HMBC.

**Table 5**: $^{1}H$ and $^{13}C$ NMR Spectral Data of Strictamine (**6**) and 11-Methoxystrictamine (**7**) [a].

| Position | 6 | | 7 | |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | - | 189.4 | - | 189.5 |
| 3 | 4.77 d (5) | 55.0 | 4.71 d (5) | 55.1 |
| 5a<br>5b | 2.63 td (14,5)<br>2.78 dd (14,6) | 51.8 | 2.60 td (14,5)<br>2.75 dd (14,6) | 51.6 |
| 6 | 2.02 dd (14,5)<br>3.73 td (14,6) | 32.5 | 1.99 dd (14,5)<br>3.67 td (14,6) | 32.0 |
| 7 | - | 55.9 | - | 55.3 |
| 8 | - | 145.9 | - | 138.0 |
| 9 | 7.43 br d (8) | 123.4 | 7.31 d (8) | 123.6 |
| 10 | 7.18 td (8,1) | 125.7 | 6.72 dd (8,2) | 111.7 |
| 11 | 7.35 td (8, 1) | 128.2 | - | 160.3 |
| 12 | 7.64 br d (8) | 121.0 | 7.20 d (2) | 107.0 |
| 13 | - | 155.3 | - | 156.8 |
| 14a<br>14a | 1.78 dd (14,3)<br>2.70 ddd (14,5,3) | 35.6 | 1.75 dd (14,3)<br>2.69 ddd (14,5,3) | 35.5 |
| 15 | 3.53 br s | 32.2 | 3.52 br s | 32.2 |
| 16 | 2.09 d (3) | 55.1 | 2.07 d (3) | 55.5 |
| 18 | 1.57 dd (7, 3) | 12.9 | 1.55 dd (7, 2) | 13.0 |
| 19 | 5.55 br q (7) | 120.8 | 5.53 br q (7) | 121.5 |
| 20 | - | 136.5 | - | 133.5 |
| 21a | 3.18 d (17) | 53.4 | 3.17 d (17) | 53.4 |
| 21b | 4.11 br d (17) | - | 4.07 br d (17) | - |
| $CO_2$-Me | 3.73 s | 51.6 | 3.72 s | 51.7 |
| $\underline{C}O_2$-Me | - | 171.4 | - | 171.4 |
| 11-OMe | - | - | 3.79 s | - |

[a]$CDCl_3$, 400 and 100 MHz; assignments based on COSY, HMQC and HMBC.

**Table 6**: $^{1}H$ Spectral Data of Quebrachidine (**10**), Vincamajine (**11**) and Vincamajine 17-*O*-veratrate (**12**)[a].

| Position | 10 | 11 | 12 |
|---|---|---|---|
| 2 | 3.82 br s | 3.22 d (5) | 3.27 d (5) |
| 3 | 3.45 m | 3.55 dd (10,5) | 3.60 dd (9,5) |
| 5 | 3.54 d (5) | 3.57 d (5) | 3.69 d (5) |
| 6a<br>6b | 1.73 d (12)<br>2.63 dd (12, 5) | 1.76 d (12)<br>2.58 dd (12,5) | 1.88 d (11)<br>2.72 d (11) |
| 9 | 7.81 br d (8) | 7.12 dd (8,1) | 6.87 br d (7) |
| 10 | 6.81 td (8,1) | 6.77 td (8,1) | 6.53 t (7) |
| 11 | 7.12 td (8, 1) | 7.14 td (8,1) | 7.11 t (7) |
| 12 | 6.78 br d (8) | 6.63 dd (8,1) | 6.67 br d (7) |
| 14a<br>14b | 1.44 dd (14, 10)<br>2.56 dd (14, 5) | 1.49 dd (14,10)<br>2.42 dd (14,5) | 1.59 td (12, 4)<br>2.72 br d (12) |
| 15 | 3.45 m | 3.46 d (5) | 3.55 d (4) |
| 17 | 4.29 s | 4.02 s | 5.91 br s |
| 18 | 1.59 dt (7,2) | 1.57 br d (7) | 1.55 d (7) |
| 19 | 5.26 br q (7) | 5.26 br d (7) | 5.29 br q (7) |
| 21a<br>21b | 3.45 m<br>3.45 m | 3.44 m<br>3.44 m | 3.50 br s<br>3.50 br s |
| N(1)-Me | - | 2.61 s | 2.68 s |
| $CO_2$-Me | 3.70 s | 3.67 s | 3.39 s |
| 2′ | - | - | 7.39 d (2) |
| 5′ | - | - | 6.88 br d (8) |
| 6′ | - | - | 7.55 dd (8, 2) |
| 3′-OMe | - | - | 3.90 s |
| 4′-OMe | - | - | 3.94 s |

[a]$CDCl_3$, 400 and 100 MHz, assignment based on COSY, HMQC and HMBC.

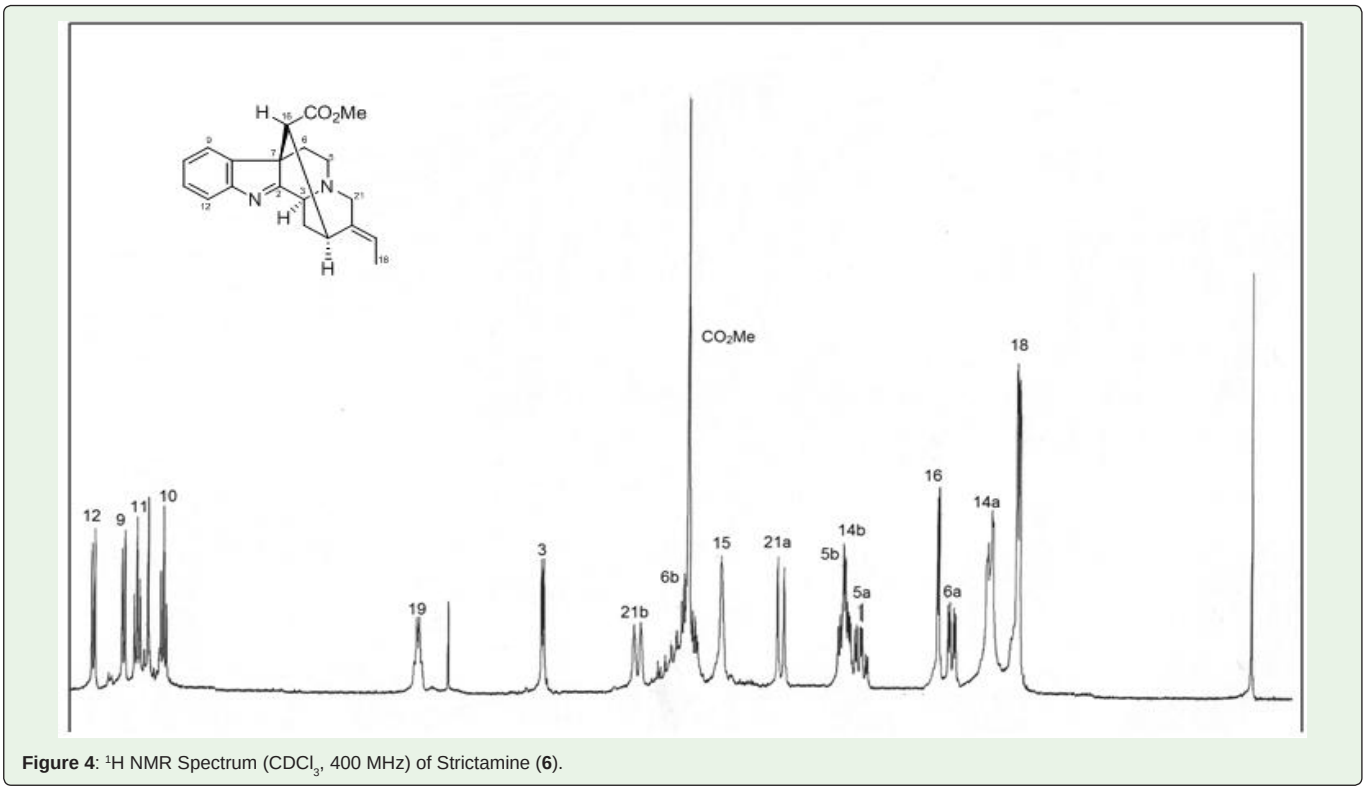



**Figure 4**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of Strictamine (**6**).

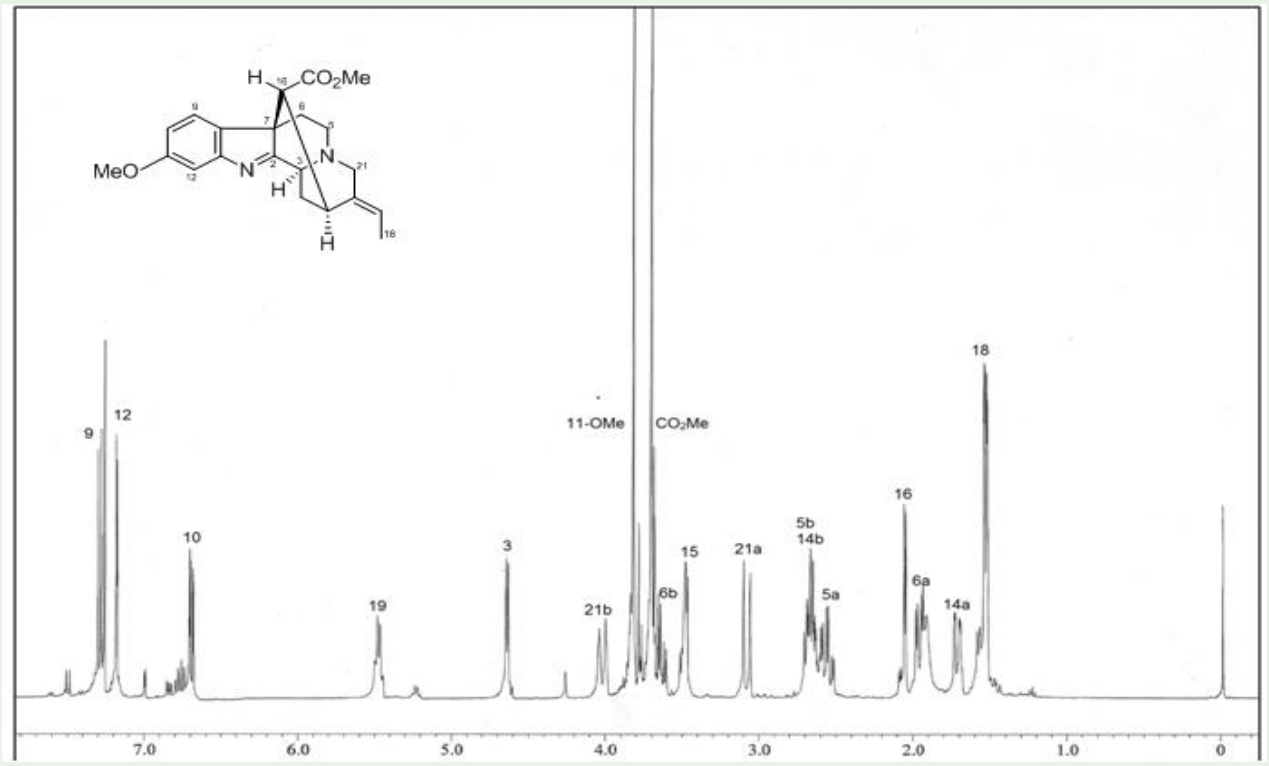



**Figure 5**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of 11-Methoxystrictamine (**7**).

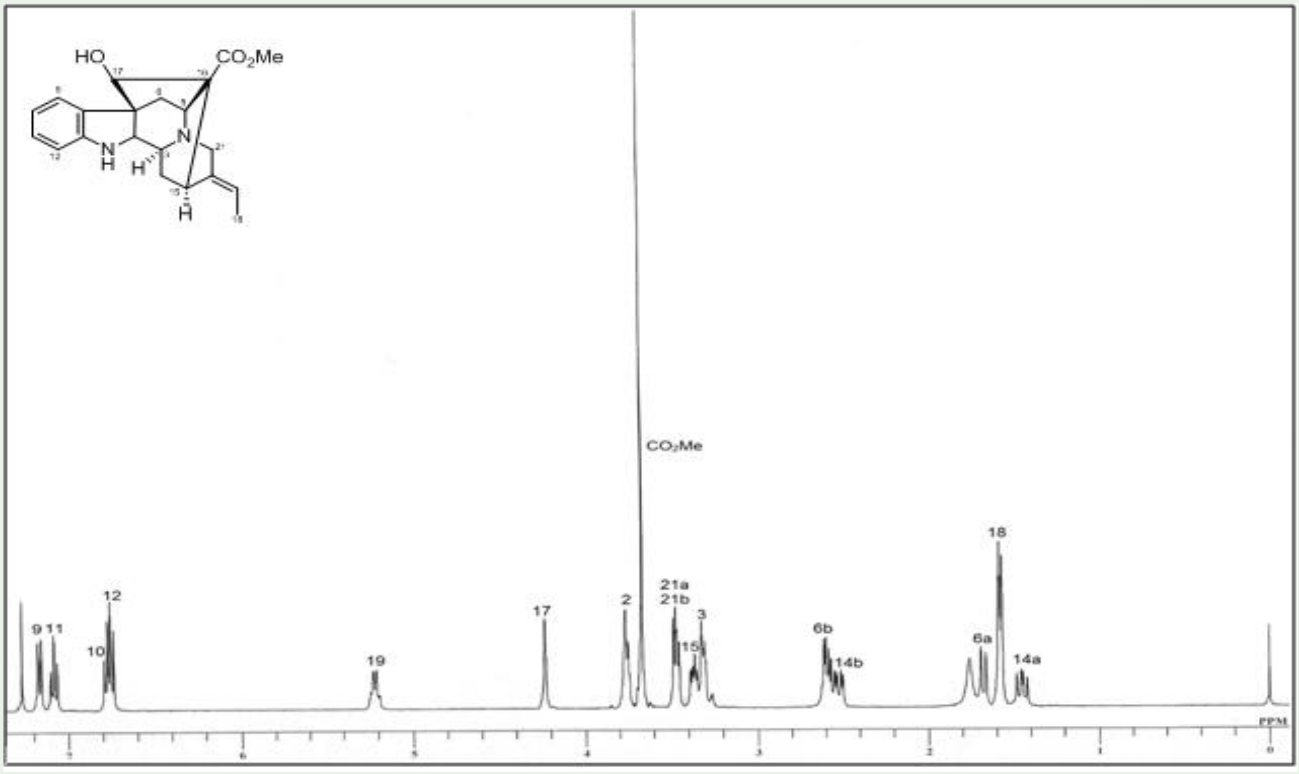



**Figure 6**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of Quebrachidine (**10**).

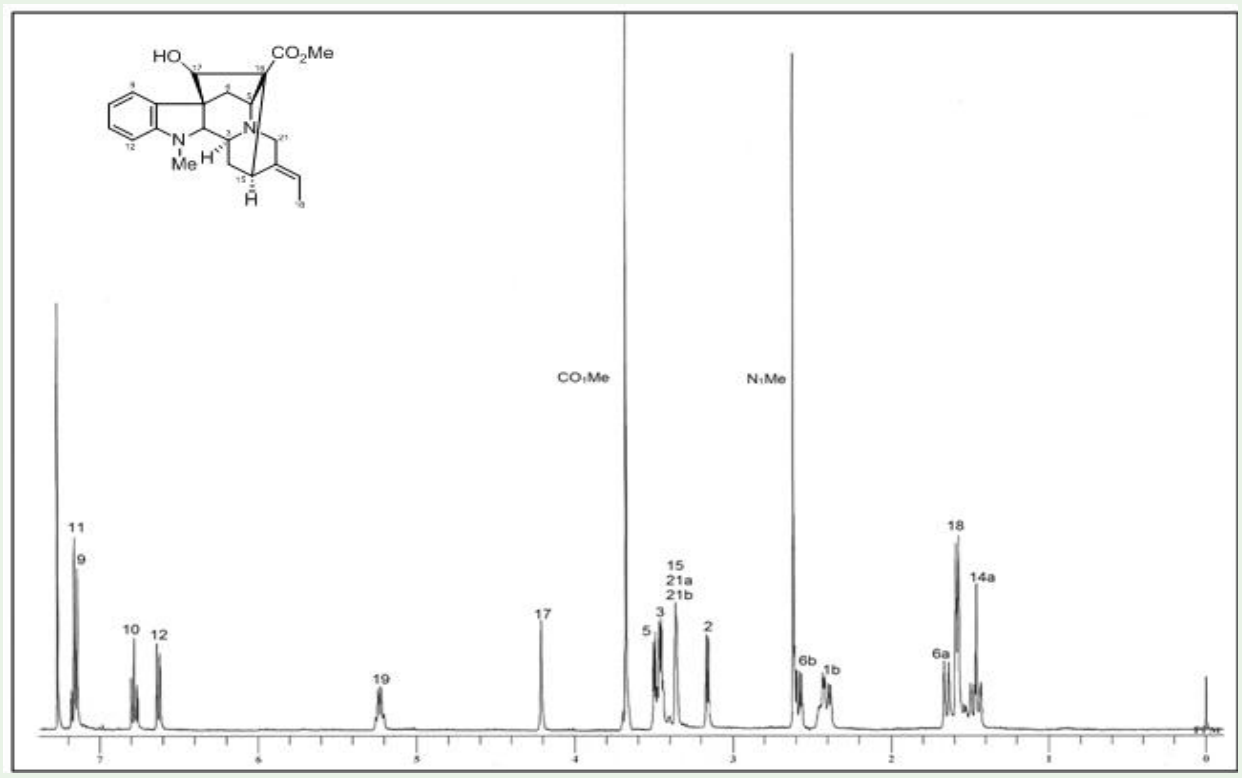



**Figure 7**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of Vancamajine (**11**).

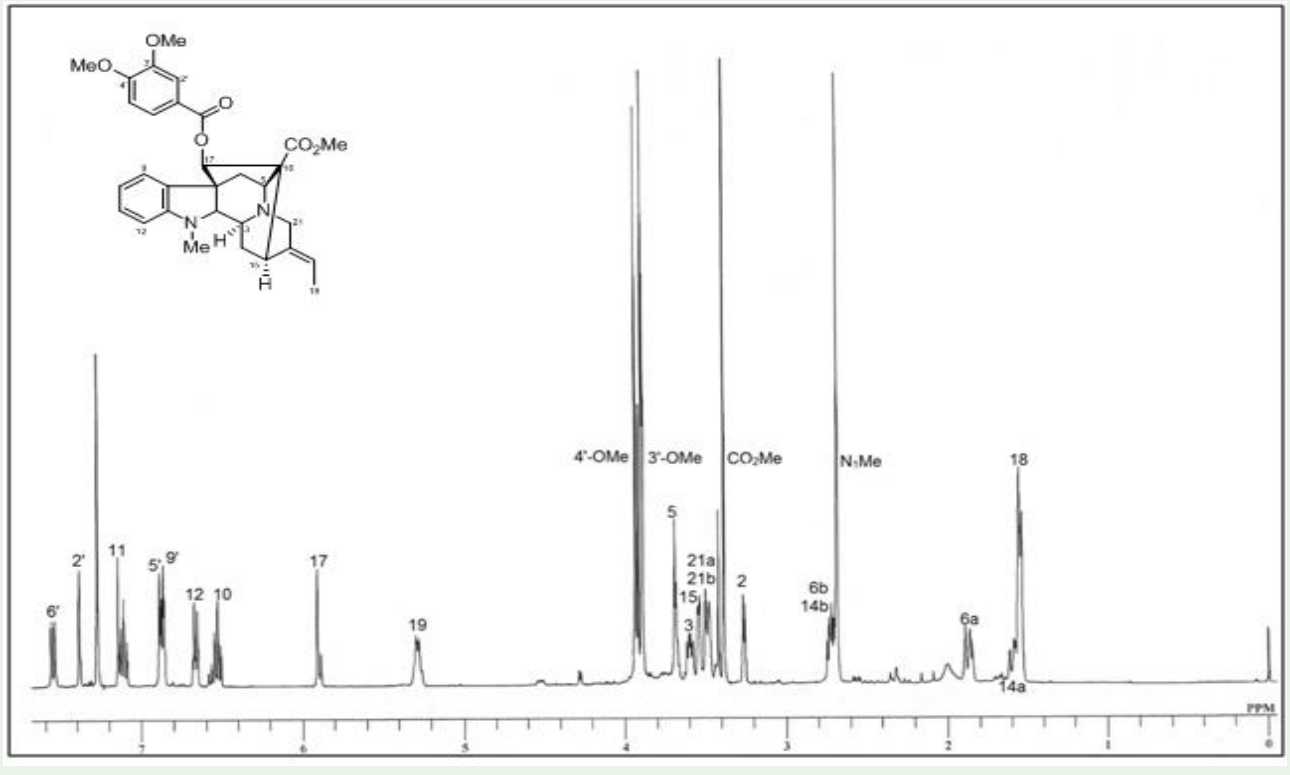



**Figure 8**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of Vincamajine 17-*O*-veratrate (**12**).

**Table 7**: $^{13}$C NMR Spectral Data of Quebrachidine (**10**), Vincamajine (**11**) and Vincamajine 17-*O*-veratrate (**12**)[a].

| Position | 10 | 11 | 12 |
|---|---|---|---|
| 2 | 68.5 | 74.4 | 74.9 |
| 3 | 54.6 | 53.1 | 53.2 |
| 5 | 61.6 | 61.6 | 61.6 |
| 6 | 35.6 | 35.2 | 36.7 |
| 7 | 57.8 | 57.0 | 56.3 |
| 8 | 129.6 | 130.1 | 128.7 |
| 9 | 124.8 | 128.2 | 123.5 |
| 10 | 119.6 | 119.1 | 119.0 |
| 11 | 128.2 | 124.4 | 128.5 |
| 12 | 110.9 | 109.0 | 109.1 |
| 13 | 151.7 | 154.4 | 154.2 |
| 14 | 22.3 | 21.8 | 21.8 |
| 15 | 30.3 | 30.0 | 30.2 |
| 16 | 59.6 | 59.4 | 59.1 |
| 17 | 74.3 | 74.5 | 74.9 |
| 18 | 12.8 | 12.8 | 12.6 |
| 19 | 116.4 | 116.5 | 116.8 |
| 20 | 136.8 | 136.5 | 136.5 |
| 21 | 55.3 | 55.2 | 55.5 |
| $CO_2$-Me | 51.6 | 51.5 | 51.6 |
| C$O_2$-Me | 173.3 | 173.0 | 172.2 |
| N(1)-Me | - | - | 34.2 |
| 1' | - | - | 122.1 |
| 2' | - | - | 111.9 |
| 3' | - | - | 148.6 |
| 4' | - | - | 153.0 |
| 5' | - | - | 110.3 |
| 6' | - | - | 123.2 |
| 3'-OMe | - | - | 55.9 |
| 4'-OMe | - | - | 55.9 |
| -C=O | - | - | 163.8 |

[a]$CDCl_3$, 400 and 100 MHz; assignments based on COSY, HMQC and HMBC.

by signal observed at $\delta$ 12.9 (C-18) and 120.8 (C-19). An amino methylene (C-21) was observed due to the carbon signal resonated at $\delta$ 53.4, while the observed carbon signals at $\delta$ 51.6 and 171.4 were due to the presence of a methyl ester group ($CO_2$Me). The $^1$H and $^{13}$C NMR of **7** (Table 5, Figure 5) were similar to that of **6**, except that **7** possess an additional methoxy group at C-11 when compared to those of **6**, as mentioned in the earlier discussion. The $^1$H NMR spectrum showed three aromatic proton resonances ($\delta$ 6.72, 7.20, and 7.31), and a methoxy group substituted at C-11 at $\delta$ 3.79 ($\delta_c$ 160.3, C-11). Therefore, compound **7** is the 11-methoxy derivative of strictamine (**6**) [14].

## Ajmaline group

**Quebrachidine (10), Vincamajine (11) and Vincamajine-17-*O*-veratrate (12):** The $^1$H NMR spectrum of **10** (Figure 6, Table 6) showed the presence of four aromatic hydrogen due to an unsubstituted indole chromophore, where four proton signals were observed at $\delta$ 6.78–7.81. The presence of the characteristic ethylidene side chain was indicated by signals observed at $\delta$1.59 (C18) and $\delta$ 5.26 (C-19). One methoxy singlet corresponded to $CO_2$Me was observed at $\delta$ 3.70. The $^{13}$C NMR (Table 7) of **10** gave a total of 21 carbon

**Table 8**: $^1$H and $^{13}$C NMR Spectral Data of Pleiocarpamine (**13**)[a].

| Position | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 2 | - | 136.8 |
| 3 | 3.84 m | 50.5 |
| 5a | 2.22 m | 49.8 |
| 5b | 3.35 ddd (13,10,3) | - |
| 6a | 2.66ddd (16,10,6) | 20.6 |
| 6b | 3.14ddd (16,9,3) | - |
| 7 | - | 107.9 |
| 8 | - | 128.5 |
| 9 | 7.53 m | 118.2 |
| 10 | 7.09 m | 119.8 |
| 11 | 7.09 m | 120.5 |
| 12 | 6.94 m | 112.2 |
| 13 | - | 137.4 |
| 14a | 2.19 ddd (13,3,2) | 28.4 |
| 14b | 2.49 ddd (13,3,2) | - |
| 15 | 3.51br s | 33.6 |
| 16 | 5.21 d (4) | 61.1 |
| 18 | 1.49 dd (7,2) | 12.4 |
| 19 | 5.30qd (7,2) | 122.7 |
| 20 | | 133.1 |
| 21a | 1.73br d (13) | 56.4 |
| 21b | 2.59 d (13) | - |
| $CO_2$-Me | 3.56 s | 51.8 |
| C$O_2$-Me | - | 169.0 |

[a]$CDCl_3$, 400 and 100 MHz; assignments based on COSY, HMQC and HMBC.

**Table 9**: $^1$H and $^{13}$C NMR Spectral Data of 11-Methoxyakuammicine (**14**) and 11- Methoxyakuammicine-*N*-(4)-oxide (**15**) [a].

| | 14 | | 15 | |
|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | - | 167.5 | - | 164.7 |
| 3 | 4.12 br s | 61.8 | 4.29 br s | 78.3 |
| 5 | 3.06 dd (12, 6)<br>3.37 td (12, 6) | 55.7 | 3.71 m<br>3.99 m | 69.9 |
| 6 | 1.87 dd (12, 6)<br>2.53 td (12, 6) | 45.6 | 1.93 dd (14, 7)<br>2.47 td (14, 7) | 41.7 |
| 7 | - | 56.5 | - | 54.1 |
| 8 | - | 128.8 | - | 126.8 |
| 9 | 7.14 d (8) | 121.2 | 7.41 d (8.5) | 121.9 |
| 10 | 6.43 dd (8, 2) | 105.5 | 6.40 dd (8.5, 2) | 106.0 |
| 11 | - | 160.3 | - | 160.9 |
| 12 | 6.43 d (2) | 97.0 | 6.37 b (2) | 97.5 |
| 13 | - | 144.5 | - | 144.1 |
| 14 | 1.34 dt (14, 3)<br>2.44 ddd (14, 3, 2) | 29.4 | 1.37 br d (14)<br>2.75 br d (14) | 27.9 |
| 15 | 3.96 br s | 30.5 | 3.97 br s | 28.5 |
| 16 | - | 101.6 | - | 102.0 |
| 18 | 1.67 dt (7,1) | 13.0 | 1.58 d (6,8) | 13.6 |
| 19 | 5.42 br q (7) | 122.4 | 5.55 br q (6,9) | 127.1 |
| 20 | - | 137.4 | - | 133.3 |
| 21 | 3.03 d (14)<br>3.98 br d (14) | 56.4 | 3.99 d (14,5)<br>4.19 d (14,5) | 74.0 |
| N(1)-H | 8.95 s | - | 8.85 s | - |
| $CO_2$-Me | - | 167.7 | 3.75 s | 51.4 |
| $CO_2$-Me | 3.81 s | 51.1 | - | 167.2 |
| 11-OMe | 3.78 s | 55.5 | 3.70 s | 55.6 |

[a]$CDCl_3$, 400 and 100 MHz; assignments based on COSY, HMQC and HMBC.

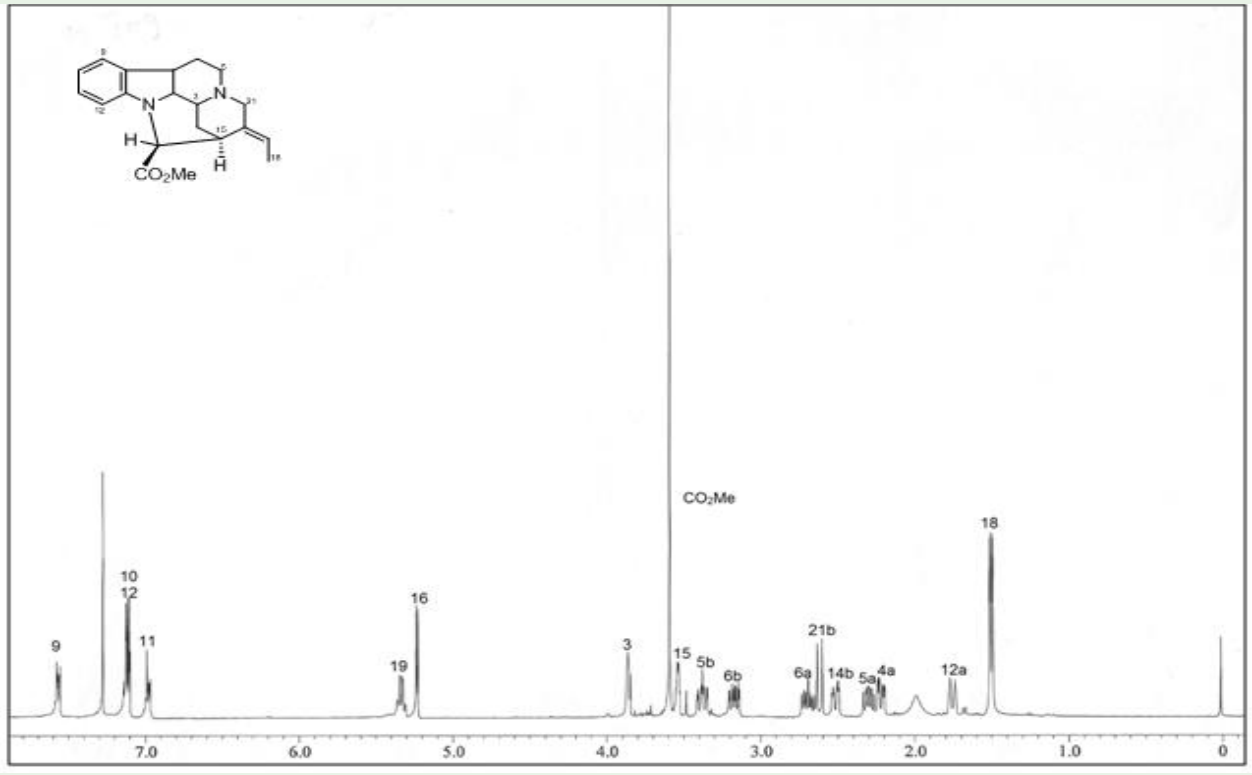



**Figure 9**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of Pleiocarpamine (**13**).

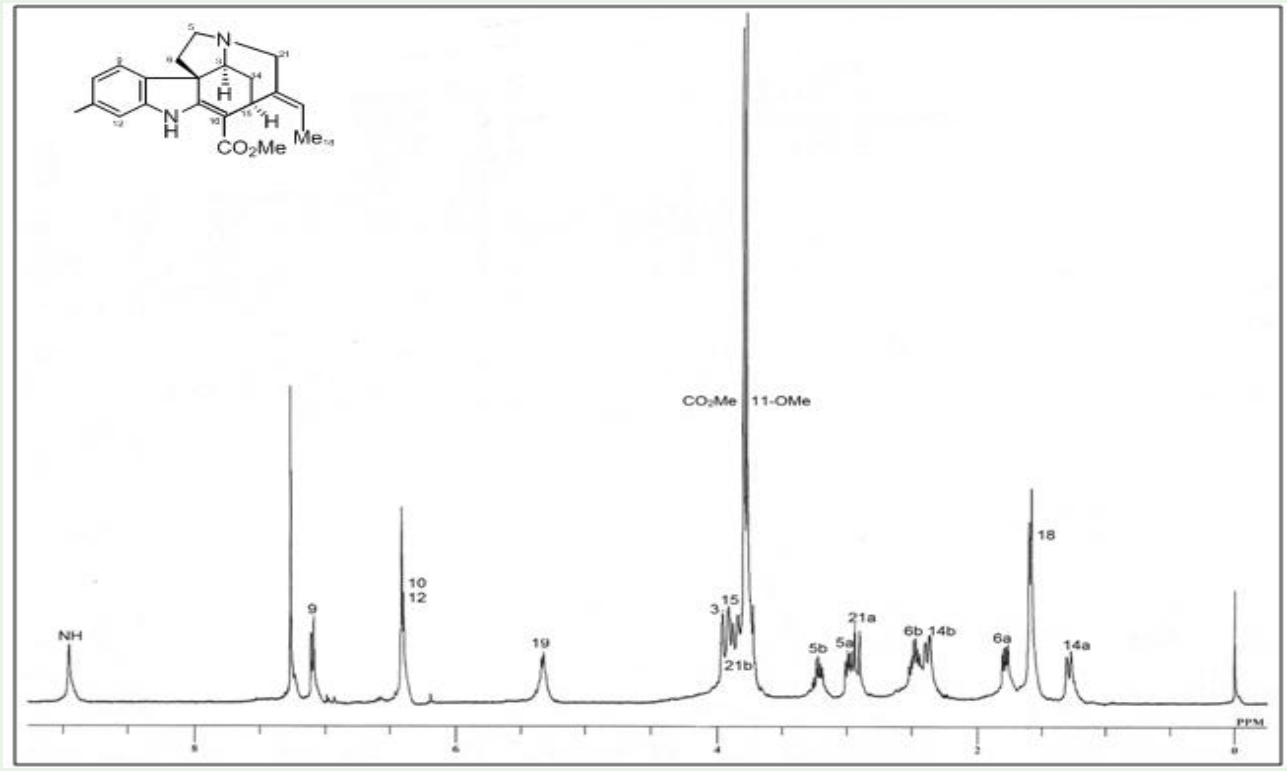



**Figure 10**: $^{1}H$ NMR Spectrum ($CDCl_3$, 400 MHz) of 11-methoxyakuammicine (**14**).

resonances (δ110.9 – 151.7). The presence of ethylidene side chain was supported by the observed carbon signals at δ 136.8 (C-20) and δ 116.4 (C-19). An amino methylene (C-21) was resonated at δ 55.3, while the observed carbon signals at δ 51.6 and 173.3 were due to the presence of a methyl ester group ($CO_2Me$). The spectra data of **11**(Figure 7) were similar to that of **10**, except for the presence of an additional singlet at $\delta_H$ 2.61, which was assigned to N(1)-Me. The $^1$H NMR spectrum of **12** (Figure 8) showed similar features as those shown by vincamajine (**11**), except for the presence of additional signals due to the acid residue (30 ,40 -dimethoxybenzoic acid or veratric acid) associated with an ester group at C-17 (vincamajine 17-O-veratrate) [15].

### Corynantheine group

**Pleoicarpamine (13):** The $^1$H NMR spectrum (Figure 9) of **13** showed the presence of four aromatic proton signals δ 6.94 – 7. 53, confirming the presence of the unsubstituted indole moiety. The presence of the characteristic ethylidene side chain was indicated by signals observed at δ 1.47 and 5.30. The $^{13}$C NMR data (Table 8) gave a total of 20 carbon resonances. The aromatic resonances observed at δ 118.2 (C-9), 119.8 (C-10), 120.5 (C-11), 112.2 (C-12) and 137.4 (C-13). The presence of a trisubstituted double bond due to the ethylidene side chain was supported by the observed carbon signals at δ 133.1 (C-20) and 122.7 (C-19). An amino methylene (C-21) was observed due to the carbon signal resonated at δ 56. 4 and δ 169.0 were due to the presence of a methyl ester group ($CO_2Me$).

### Strychnan group

**11-Methoxyakuammicine (14) and 11-methoxyakuammicine-*N*-(4)-oxide (15):** The $^1$H NMR spectrum of **14** (Figure 10) showed signals due to three aromatic hydrogen observed in range at δ 6.43-7.41 (H-10, H-12) and 7.14 (H-9). Two strong absorption singlet peak at δ 3.78 and δ3.81 were assigned to the 11-OMe substitution at indole ring and methyl group present in the $CO_2Me$ substituted group respectively. It also revealed the presence of the *N*(1)-H group at δ 8.95 as a singlet, in addition to the presence of an ethylidene side chain observed at δ 1.67 (H-18) and 5.42 (H-19). The $^{13}$C NMR data (Table 9) of **14** gave a total of 21 carbon resonances. The methoxy group at C-11 was confirmed by the carbon resonances observed at δ160.3. The presence of the ester substituted group confirmed by the signals observed at δ 167.7 and 51.1. The presence of the ethylidene side chain was supported by the observed carbon signals at 137.4 (C-20) and 122.4 (C-19). Compound **15** was readily identified as the N4-oxide of **14** from its NMR data (Table 9, Figure 11), in particular the characteristic downfield shifts of the carbon resonances for C-3, C-5, and C-21, when compared with those of **14**.

## Conclusion

A comparison of the present results with that of an earlier study of the alkaloids collected from the East Coast, Terengganu [16] and West Coast, Perak [17] of Peninsular Malaysia is presented. Alkaloids collected in this study are similar with West Coast, Perak of Peninsular Malaysia; but significantly varied with East coast sample, whereby eight alkaloids (compound 6 - 12 and 15) were not reported in East Coast, Terengganu's studies. Although 15 alkaloids in this study are known compounds, however, antimicrobial and pharmacological effects of each pure compound have not been reported in literature. Except Compound 4 has been reported to show potent vasorelaxant activity [18] and Compound 6 had been shown moderate activity in vincristine-resistant human oral epidermoid carcinoma cell cell line [17]. In future, alkaloids collected may further analysis of its

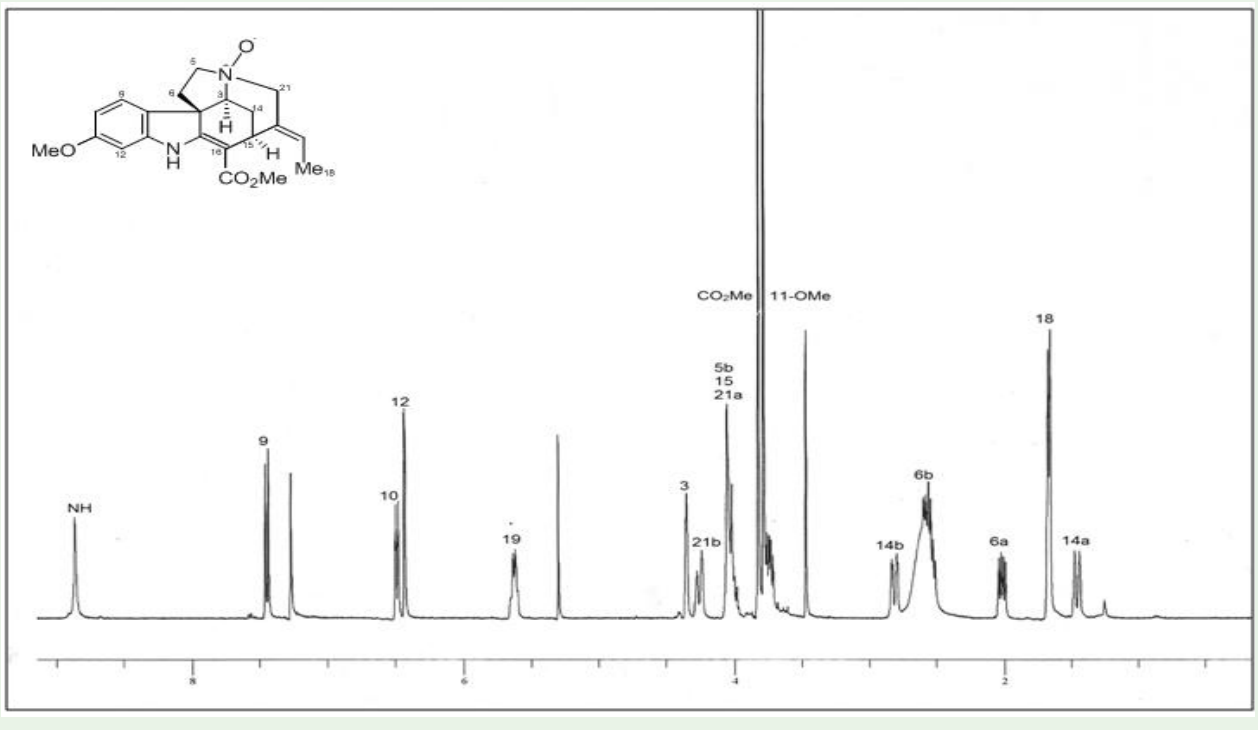



**Figure 11**: $^1$H NMR Spectrum ($CDCl_3$, 400 MHz) of 11-methoxyakuammicine-*N*-(4)-oxide (**15**).

pharmacological effects such as antimicrobial activities, antioxidant activities and anti-inflammatory activities.

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