

## CHAPTER III

### EXPERIMENTAL

#### 1. Source of Plant Materials

The leaves of *Ancistrocladus tectorius* (Lour.) Merr. were obtained from Sakae-Raj Environmental Research Station, Pak Thong Chai District, Nakhon Ratchasima Province, Thailand in October, 1982. The plant materials were authenticated by comparison with the specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. A herbarium specimen is deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

#### 2. General Techniques

##### 2.1 Thin-layer Chromatography (TLC)

###### Analytical

- Technique : one way, ascending
- Adsorbents : silica gel G (E.Merck) 15 g + silica gel GF<sub>254</sub> (E.Merck) 15 g/60 ml distilled water.  
aluminium oxide G (E.Merck) 35g + aluminum oxide GF<sub>254</sub> (E.Merck) 35 g/80 ml distilled water.
- Plate size : 10 cm x 20 cm and 20 cm x 20 cm

Layer thickness : 250  $\mu$

Activation : air dried for 15 minutes and then at 110°C for  
1 hour.

Solvent system : a) silica gel (G + GF)/anaesthetic ether  
b) silica gel (G + GF) / chloroform : acetone  
(1:9)  
c) aluminium oxide (G + GF)/anaesthetic ether  
d) aluminium oxide (G + GF)/chloroform  
e) aluminium oxide (G + GF)/benzene : Chloroform  
(4:1)

Distance : 15 cm

Laboratory temperature : 24-30°C

Detection of alkaloid on chromatographic plate

: 1) UV light of 254 nm

The alkaloids become visible as quenching spots on the yellowish green fluorescent background plate in uv light of 254 nm.

2) Dragendorff 's spray reagent

Solution A : bismuth subnitrate (850 mg),  
distilled water (40 ml) and acetic acid  
(10 ml)

Solution B : potassium iodide (8 g) and  
distilled water (20 ml)

Solution A and B, 5 ml each, were mixed,  
20 ml of glacial acetic acid and 70 ml  
of distilled water were added and used as  
spray reagent. The alkaloids give

orange or yellow orange spots with Dragendorff's reagent.

## 2.2 Column Chromatography (CC)

- Column size : 6 cm x 20 cm
- Adsorbent : aluminium oxide neutral, 0.063-0.200 mm  
(E. Merck)
- Packing of column : dry packing
- Addition of alkaloidal material to column : the portion of crude alkaloid was dissolved in small amount of organic solvent, mixed with small quantity of adsorbent, air dried, triturated and added onto the top of a dry column.
- Solvent : diethyl ether
- Collection of eluate : fractions of 20 ml were collected.
- Examination of eluate : those fractions giving on orange colour with Dragendorff's spray reagent were examined by thin layer chromatography.

## 2.3 Physical Constants

- Melting Points : Melting points were determined by heating stage microscope (Reichert Melting Point Apparatus).
- Specific Rotation : Specific rotation were obtained in chloroform with a Perkin Elmer Model 241 Polarimeter.

### Circular Dichroism (CD) spectrum

: CD spectrum was obtained in methanol with a Jasco Model J-40A Automatic Recording Spectropolarimeter.

## 2.4 Spectroscopy

### Ultraviolet (UV) Absorption spectrum

Ultraviolet absorption spectrum was obtained with a Shimadzu Double-Beam Spectrophotometer.

### Infrared (IR) Absorption Spectrum

Infrared absorption spectrum was obtained with a Perkin-Elmer Model 283 Spectrophotometer, absorption bands are reported in wave number ( $\text{cm}^{-1}$ ).

### Nuclear Magnetic Resonance (NMR) Spectra

Proton nmr spectra were recorded on a Nicolet NT-360 instrument operating at 360 MHz at the NSF Regional nmr Facility at the University of Illinois at Urbana, Urbana Champaign. Tetramethylsilane (T.M.S.) was used as an internal standard and chemical shifts were reported on the ppm scale.

The nOe experiments were set up to assign the  $^1\text{H}$  nmr spectrum completely.

### Mass Spectra

Mass spectra were obtained with Finnegan Model 4500 Quadrupole Mass Spectrometer.

Electron impact (e.i.) mass spectrum was determined at 70 eV with inlet temperature 180°C

Chemical ionization (c.i.) mass spectrum was obtained at 70 eV using methane (CH<sub>4</sub>) as the reagent gas.

### Single Crystal X-ray Crystallographic Analysis

The X-ray intensity data were measured with Mo-K<sub>α</sub> radiation on a Nicolet P3 Automated Diffractometer.

### 3. Extraction and Isolation of Alkaloid(s) from *Ancistrocladus tectorius* (Lour.) Merr. Leaves.

#### 3.1 Extraction of Crude Alkaloid

The dried, coarsely powdered leaves (800 g) were macerated twice for 3 day periods with 95% ethanol (8 liters and 5 liters). The ethanol extracts were combined and evaporated under reduced pressure. The residue (125 g) was suspended in distilled water (5 liters) and the filtrates extracted with chloroform (2 x 5 liters). The combined chloroform extracts were dried over anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>), evaporated under reduced pressure to afford a light brown powder of crude alkaloid (13 g). The crude alkaloid contained at least two alkaloids as indicated by thin layer chromatography (Figure 7, p. 61)

#### 3.2 Isolation of Alkaloid AT-1

The powder of crude alkaloid was divided into six portions. Each portion was separately chromatographed by dissolving in chloroform (10 ml), absorbing onto neutral alumina (5 g), air dried, triturated, placed on the top of a dry alumina column (6 cm x 20 cm)

and then eluted with diethyl ether. Twenty fractions were collected, examined by TLC (alumina (G + GF)/anaesthetic ether) and fractions of similar composition were combined. Pooled fractions 9-20 were homogeneous by TLC, crystallised from acetone to yield pale yellow needles (125 mg, 0.016% yield). This was designated as AT-1 and was subsequently named as ancistrotoectorine.

#### 4. Characterisation of Ancistrotoectorine

Ancistrotoectorine was obtained as pale yellow needles. It was soluble in chloroform, ether, acetone and ethanol.

##### 4.1 hRf Values

The hRf values given are those obtained with the following systems :

- a) silica gel (G + GF)/anaesthetic ether = 9.4
- b) silica gel (G + GF)/chloroform : acetone (1:9) = 22.7
- c) aluminium oxide (G + GF)/anaesthetic ether = 69.3
- d) aluminium oxide (G + GF)/chloroform = 75.6
- e) aluminium oxide (G + GF)/benzene : chloroform (4:1) = 28.9

The thin layer chromatograms of ancistrotoectorine (AT-1) are shown in Figures 8-12, pp. 62-66.

##### 4.2 Melting Point

134-140°C (uncorrected)

##### 4.3 Specific Rotation

$$[\alpha]_D^{26} = 0^\circ \quad (\text{CHCl}_3)$$

#### 4.4 Circular Dichroism (MeOH)

$$[\theta]_{218} = + 70,167$$

$$[\theta]_{234} = - 34,179$$

$$[\theta]_{283} = - 10,291$$

$$[\theta]_{308} = - 5,146$$

$$[\theta]_{321} = - 6,549$$

$$[\theta]_{336} = - 8,420$$

(Figures 13, p. 67)

#### 4.5 Molecular Weight

$$(M^+ + H^+) = 422 \text{ (c.i. mass spectrometry)}$$

#### 4.6 Ultraviolet Absorption Spectrum

The  $\lambda_{\text{max}}$  (EtOH) was at 234 ( $\log \epsilon = 4.73$ ), 286 ( $\log \epsilon = 3.64$ ), 306 ( $\log \epsilon = 3.61$ ), 323 ( $\log \epsilon = 3.66$ ) and 337 nm ( $\log \epsilon = 3.69$ ) as shown in Figure 14, p. 68).

#### 4.7 Infrared Absorption Spectrum (Potassium Bromide Disc)

$$\nu_{\text{max}} \text{ (cm}^{-1}\text{)}$$

3375, 2960, 2925, 2840, 2770, 1640, 1600, 1555, 1458,  
1400, 1362, 1320, 1115, 1095, 1085, 825 and 755.

(Figure 15, p. 69).

#### 4.8 NMR Spectra

In deuteriochloroform at 360 MHz in  $\delta$  values (ppm)  
from tetramethylsilane (T.M.S.)

Proton	Multiplicity	Chemical Shifts ( $\delta$ )	Coupling Constants
3-CH <sub>3</sub> (3H)	d	1.268	$J = 6.1$ Hz
1-CH <sub>3</sub> (3H)	d	1.466	$J = 6.4$ Hz
2'-CH <sub>3</sub> (3H)	s	2.169	
N-CH <sub>3</sub> (3H)	s	2.484	
3-H (1H)	ddq	2.544	$J = 3.0, 6.1, 10.3$ Hz
4-H <sub><math>\beta</math></sub> (1H)	dd	2.655	$J = 3.0, 15.3$ Hz
4-H <sub><math>\alpha</math></sub> (1H)	dd	2.787	$J = 10.3, 15.4$ Hz
8-OCH <sub>3</sub> (3H)	s	3.323	
6-OCH <sub>3</sub> (3H)	s	3.691	
1-H (1H)	q	3.731	$J = 6.4$ Hz
5'-OCH <sub>3</sub> (3H)	s	3.985	
5-H (1H)	s	6.518	
6'-H (1H)	d	6.689	$J = 7.5$ Hz
1'-H (1H)	s	7.232	
7'-H (1H)	t	7.266	$J = 7.8$ Hz
8'-H (1H)	d	7.343	$J = 8.0$ Hz
4'-OH (1H)	s	9.565	

<sup>1</sup>H nmr spectra and nOe experiments of alkaloid AT-1 are shown in Figures 16-26, pp. 70-80

#### 4.9 Mass Spectra

e.i. mass spectrum :

m/z (%) no M<sup>+</sup> observed, 420 (1), 407 (28), 406 (100), 360 (17), 203 (72), 202 (5), 195 (6), 189 (4), 188 (9), 187 (5), 181 (8), 180 (36), 172 (28), 167 (9), 165 (28), 158 (31), 151 (33), 145 (8), 144 (8), 143 (8), 137 (7), 136 (8), 131 (7), 129 (10), 128 (8), 115 (12) and 107 (7).



c.i. mass spectrum :

m/z (%) 422 ( $M^+ + H^+$ , 100) and 406 (9).

#### 4.10 Crystallographic Analysis

Crystal data  $C_{26}H_{31}NO_4$ ,  $M = 421.5$ , monoclinic,

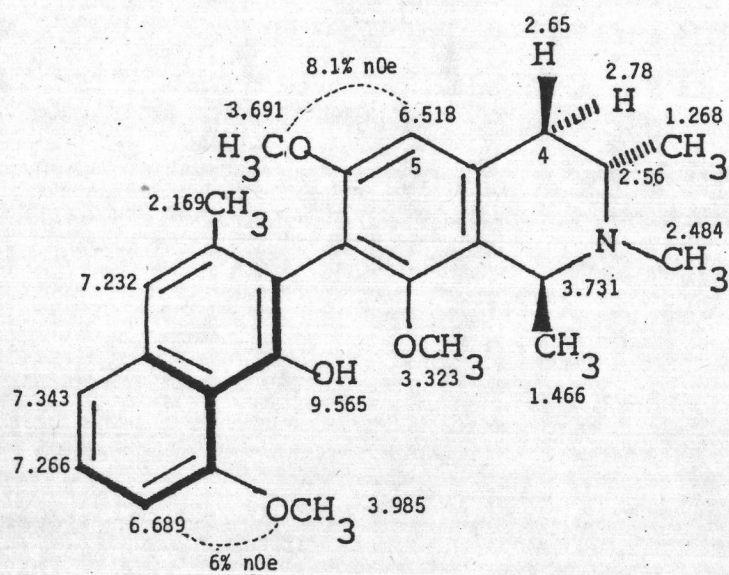
$a = 11.858(14)\text{\AA}$ ,  $b = 7.043(7)\text{\AA}$ ,  $c = 14.693(28)\text{\AA}$ ,

$\beta = 111.43(12)^\circ$ ,  $V = 1142.6\text{\AA}^3$ ,  $Z = 2$ ,  $D_c = 1.22\text{ g}\cdot\text{cm}^{-3}$ ,

space group  $P2_1$ ,  $\mu(\text{Mo-K}\alpha) = 0.46\text{ cm}^{-1}$

The crystal structure was elucidated using MULTAN and refined using SHELX<sup>(45,46)</sup>. In the final cycles of leastsquares refinement the positional parameters for all atoms, anisotropic thermal parameters for the C and O atoms and common isotropic thermal parameters for the methyl and non-methyl hydrogens were varied. These parameters are listed in pp. 85-92.

From the spectral data and crystal data obtained, AT-1 was characterised as a new 7-3' linkage naphthalene-isoquinoline alkaloid. This alkaloid is regarded as a second member of this group of alkaloid, named Ancistrotectorine. The structure of which is shown in p. 45.



Structure of Ancistrotoecorine

(Stereochemistry omitted)