CHAPTER III

EXPERIMENTAL

1. Source of Plant Material

The leaves of *Pluchea indica* Less. (Compositae) used in this study were obtained from Nakornpathom, Thailand during April-May, 1981. The plant material was authenticated by comparison with voucher specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand.

2. General Techniques

2.1 Thin Layer Chromatography (TLC)

Analytical

Technique	: one way, ascending
Adsorbent	: silica gel G (E. Merck), calcium sulphate binder 13% ; 30 g/60 ml of distilled water
Plate size	: 20 cm x 20 cm, 10 cm x 20 cm
Layer thickness	: 0.25 mm
Activation	: air dried for 15 minutes and then heating in an oven at 110 °C for one hour
Solvent systems	: a) anaesthetic ether b) chloroform : acetone (9:1) c) chloroform : ethyl acetate (1:4)

Distance : 15 cm

Laboratory temperature : 25-30 °C

Detection : 2% methanolic solution of resorcin mixed with 2% sulphuric acid solution (1:1) / heating at 110 °C for 10 minutes

Result : orange colour for PI-1 and pink colour for PI-2

2.2 Column Chromatography (CC)

Adsorbent : silica gel 0.040-0.063 mm (E. Merck)

Packing : dry packing

2.3 Melting Point

Melting point was determined on a heating stage microscope (Reichert) and was uncorrected.

2.4 Ultraviolet Absorption Spectrum

Ultraviolet absorption spectrum was measured on a Pye Unicam SP 1800 recording spectrophotometer.

2.5 Infrared Absorption Spectrum

Infrared absorption spectrum was obtained on a Perkin Elmer model 283 spectrophotometer, absorption bands are reported in wave numbers (cm^{-1}).

2.6 Nuclear Magnetic Resonance (NMR) Spectra

Proton NMR spectra were recorded at 60 MHz on a Varian model T-60A instrument, equipped with a Nicolet model TT-7 Fourier Transform attachment and at 400 MHz Bruker instrument. Tetramethylsilane was used as an

internal standard and chemical shifts were reported on the ppm scale.

2.7 Mass Spectrum

Mass spectrum was obtained with a Varian MAT 112 S double-focussing spectrometer operating at 70 eV.

3. Isolation of Chemical Substances from *Pluchea indica* Less. Leaves

3.1 Extraction

Powdered fresh leaves (50 kg) were macerated twice for 2 day-period each with 95% ethanol (70 l and 50 l). The ethanol extracts were combined, the alcohol removed under reduced pressure, and the residue suspended in 10 litres of warm 10% ethanol and filtered. The filtrate was treated with a 5% aqueous lead acetate solution until no further precipitation occurred. Further filtration then afforded a clear yellow solution which was extracted with chloroform (80 x 500 ml). The combined chloroform extract was dried over anhydrous sodium sulphate, evaporated under reduced pressure to afford a yellow syrupy mass (5.5 g).

3.2 Isolation of Chemical Compounds

The chloroform-soluble fraction (5.5 g) was divided into 11 portions and each portion was dissolved in chloroform (2 ml), mixed with a small amount of silica gel, dried and then placed on a dry silica gel column (2.5 x 40 cm). It was eluted with anaesthetic ether, 15 ml of

fractions were collected. Comparison of fractions by thin layer chromatography, and combination of those having similar patterns yielded three major fractions as follows :- (Fig. VI Page 83)

i. Fractions 1-5 after evaporation containing no residue.

ii. Fractions 6-10 shown by thin layer chromatography to contain one major and at least two minor components. It was evaporated under reduced pressure to dryness yielded brown syrupy mass (50 mg). This was rechromatographed in the same manner, using chloroform : ethyl acetate, 5:1 as eluent. The homogeneous fractions (7-10) were combined and evaporated under reduced pressure to yield yellow gum (30 mg). This was designated as PI-2. This compound has not been further investigated.

iii. Fractions 11-14 were shown by thin layer chromatography to be homogeneous and furnished colourless prisms (110 mg) on standing. This was designated as PI-1 and was subsequently identified as 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone. (Fig. VI Page 83)

4. Characterisation of PI-1

PI-1 was characterised by studies on melting point and ultraviolet, infrared, nuclear magnetic resonance and mass spectra.

PI-1 was obtained as colourless prisms. It was soluble in acetone, anaesthetic ether, chloroform and ethanol.

hRf values

a) 56.2 (Fig. VII Page 84)

b) 55.4 (Fig. VIII Page 85)

c) 59.0 (Fig. IX Page 86)

Melting point

165 °C

Molecular weight

452 (mass spectrometry)

Ultraviolet absorption spectrum

The λ_{max} (EtOH) at 258 nm it was apparent that the ketone was α,β,β-trisubstituted (Calc. 254 nm).Fig. X Page 87

Infrared absorption spectrum

λ_{max}	3420 cm^{-1}	-OH stretching
	2960, 2940, 2890 cm^{-1}	absorption due to -CH- group
	1740 cm^{-1}	the characteristic of the stretching vibration of -C(=O)-
	1660, 1585, 1445 cm^{-1}	ring -C-C- stretch
	1390, 1370 cm^{-1}	
	1240 cm^{-1}	acetate -C-C(=O)-O stretch
	1200, 1120, 1070, 1020 cm^{-1}	-C-O- stretching of saturated ester
	940,870 cm^{-1}	
	750 cm^{-1}	out of plane C-H bend
	620 cm^{-1}	out of plane C=C bend

(Fig. XI Page 88)

Nuclear Magnetic Resonance Spectrum

The complete assignment of the 400 MHz proton nmr spectrum ($CDCl_3$) in δ value (ppm) from tetramethylsilane (T.M.S.) Fig. XII Page 89

Chemical Shift (δ)	Proton	Multiplicity	Coupling Constants
0.98	14(3H)	bd, s	
1.26	4'(3H)	d	
1.28	15(3H)	s	
1.33	1β(H)	dt	J= 3,3,15 Hz
1.49	1α(H)	bd, ddd	J= 3,12,15 Hz
1.66	5'(3H)	s	
1.81	2β(H)	m	
1.84	2α(H)	m	
1.86	13(3H)	s	
1.92	5α(H)	dd	J= 4,13 Hz
2.09	2(OAc)(6H)	s	
2.10	12(3H)	s	
2.17	9α(H)	d	J= 15 Hz
2.17	6β(H)	dd	J= 13,15 Hz
2.25	9β(H)	d	J= 15 Hz
3.01	6α(H)	dd	J= 4,15 Hz
5.02	3β(H)	t	J= 3 Hz
5.24	3'(H)	q	J= 6 Hz

Protons are identified by the labelling scheme shown for the structure (Fig. V Page 61). Integrated signal areas are in accordance with the number of proton

assigned to them. Coupling constants derived from observed signal multiplicities are reported.

Mass spectrum

m/e 452(M^+, 1%, $C_{24}H_{36}O_8$), 434(2), 218(8), 217 (47, $C_{15}H_{21}O$), 216(26), 201(11), 193(5), 173(5), 159(8), 149(6), 131(22, $C_6H_{11}O_3$), 123(9), 121(8), 109(8), 97(10), 95(15), 83 (12), 81(13), 71(16), 69(19), 67(12), 56 (27), 55(25) and 43(100) Fig. XVI Page 93

Hydrolysis of PI-1

A solution of PI-1 (11.4 mg) in anhydrous methanol (10 ml) was added a small quantity of sodium carbonate. After 2 hours at room temperature the solvent was removed *in vacuo*, cold distilled water (10 ml) was added and the mixture extracted with chloroform (5 x 10 ml), washed with distilled water until neutral, dried over anhydrous sodium sulphate and evaporated *in vacuo* to afford a gummy mass (4.4 mg).

Ultraviolet absorption spectrum

λ_{max} (EtOH) 258 nm (log ϵ 4.03)

Nuclear magnetic resonance spectrum

The assignment of the 60 MHz nmr ($CDCl_3$) (Mild hydrolysis of PI-1)

Chemical Shift (δ)	Proton	Multiplicity
0.93	15(3H)	s
1.21	14(3H)	s

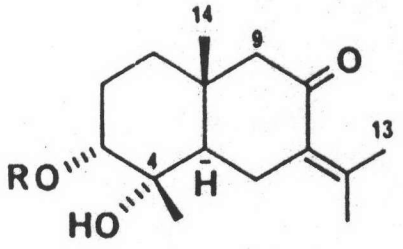
Chemical Shift (δ)	Proton	Multiplicity
1.81	13(3H)	s
2.01	12(3H)	s
2.19	9(2H)	m
2.67	3-OH(1H)	brs
2.88	4-OH(1H)	brs
3.01	6α(1H)	m
3.65	3(1H)	brs

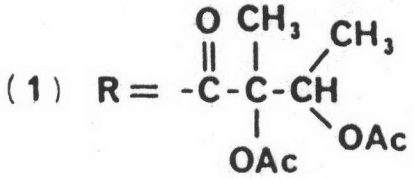
Mass spectrum

m/e 252(M^+, 6), 201(11), 194(20), 178(6), 177 (4), 175(4), 166(6), 165(6), 152(17), 151 (11), 149(9), 137(9), 125(21), 124(15), 123 (11), 121(11), 110(13), 109(14), 95(11), 84 (14), 83(14), 81(12) and 73(64)

These physical data are in agreement with those obtained previously for cuauhtemone (2) (Nakanishi *et al.*, 1974) and identity was confirmed by comparison (tlc, ms) with an authentic sample kindly supplied by Professor K. Nakanishi, Department of Chemistry, Columbia University, New York.

From the spectral data obtained and the hydrolysis product study, PI-1 was characterised as 3-(2',3'-diacetoxy-2'-methyl butyryl)-cuauhtemone (1). The structure of which is shown in Fig. V Page 61





(2) R= H

Fig. V