



## CHAPTER IV

## RESULTS AND DISCUSSION

Characterization and Identification of Isolated Alkaloid

L-1 : This compound (600 mg, 11.5 mg %) was pale yellow clear oil. L-1 was very miscible with chloroform and methanol.

M.W. : 401 (EIMS)

TLC : Rf values and results of detections were summarized as follow.

Rf value	0.85	in solvent system	A
	0.92	in solvent system	B
	0.93	in solvent system	C
	0.89	in solvent system	D
	0.88	in solvent system	E

- i fluorescent at UV 254 nm.
- ii brown spot (positive result) with iodine vapour.
- iii orange spot (positive alkaloidal result) with Dragendorff's reagent.
- iiii quenching (secondary amine) with fluorescamine.

UV :  $\lambda_{\text{max}}$  (in  $\text{CHCl}_3$ ) = 242, 274, 281 nm.

: (figure 4)

IR : (figure 5)

$\nu_{\max}$ (cm <sup>-1</sup> ) = 3,300	N-H Stretching
2,900, 2850	C-H stretching of CH <sub>3</sub> , CH <sub>2</sub>
1,720	C=O stretching of ester
1,710	C=O stretching of ketone
1,600, 1,580, 1520	aromatic C=C stretching
1,450	C-H bending (asymmetric)
1,360	C-H bending (symmetric)
1,280	C-O stretching of ester

M.S. : EIMS (figure 6)

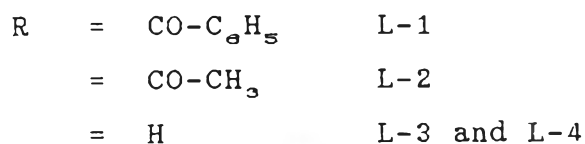
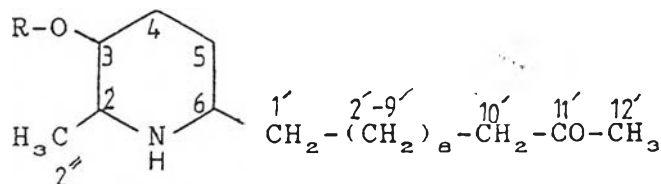
m/e (%relative) = 402(M+1)(0.87), 401(M <sup>+</sup> )(1.67),
386(1.96), 344(7.65), 296(1.98),
280(11.05), 279(33.86), 236(4.03),
218(100.0), 166(1.76), 152(3.72),
138(4.70), 124(4.99), 122(3.05).

<sup>1</sup>H-NMR : (figure 7a)

<u>chemical shift</u>	<u>multiplicity</u>	<u>No. of proton (remark)</u>
1.19	doublet*	3H (CH <sub>3</sub> at C-2)
1.27	singlet	18H (CH <sub>2</sub> of side chain)
2.12	singlet	3H (CH <sub>3</sub> adjacent to C=O)
2.42	triplet	2H (CH <sub>2</sub> adjacent to C=O)
2.83	multiplet	1H (H at C-6)
3.39	multiplet	1H (H at C-2)
5.07	multiplet	1H (H at C-3)
7.49	multiplet	3H (aromatic ring)
8.06	doublet, doublet	2H (aromatic ring)

(\* confirmed by irradiation of H-2)

$^{13}\text{C}$ -NMR : (figure 8, table 4;page 47)



Confirmation of structure and stereochemistry of L-1 by alkaline hydrolysis, can divide hydrolysed products into 2 parts:

1) Nonpolar part (D-1) which can be extracted by chloroform. The IR, MS and  $^1\text{H}$ -NMR spectra of D-1 were obtained from following datas.

IR of D-1 :  $\nu_{\text{max}}$  = 3,400, 2,950, 2,850, 1,710, 1,470,  
(figure 9) and 1,360  $\text{CM}^{-1}$ .

MS of D-1 : m/e (%rel.) = 298 ( $\text{M}^+ + 1$ ) (0.59), 297 ( $\text{M}^+$ ) (2.1),  
(figure 10) 282 (1.79), 240 (5.92), 212 (0.73)  
and 114 (100.0).

$^1\text{H}$ -NMR of D-1 : = 1.10 (d, 3H), 1.27 (s, 18H), 2.12 (s, 3H),  
(figure 11) 2.42 (t, 2H), 2.84 (m, 1H), 3.14 (m, 1H)  
and 3.64 (singlet, w1/2=20 Hz, 1H) ppm.

According to these informations, it can be identified D-1 to be iso-6-cassine

Table 4. Carbon-13 NMR Shift of L-1, L-2, L-3 and L-4.

Carbon No.	L-1	L-2	L-3	L-4
C(2'')	14.48	14.49	18.47 (18.7)	14.42 (15.7)
C(2)	48.70	48.75	56.85 (57.2)	50.77 (50.4)
C(3)	72.64	71.99	67.16 (68.0)	68.89 (68.9)
C(4)	29.60	29.63	31.90 (32.1)	27.35 (28.7)
C(5)	26.70	26.23	28.90 (29.8)	27.35 (28.7)
C(6)	48.70	48.16	55.48 (55.8)	49.10 (49.5)
C(1')	34.56	34.22	36.71 (37.0)	34.26 (34.0)
C(2')	29.20	29.20	29.20 (29.1)	29.30 (-)
C(3')	29.20	29.20	29.20 (29.1)	29.20 (-)
C(4')	29.20	29.20	29.20 (29.1)	29.30 (-)
C(5')	29.20	29.20	29.20 (29.1)	29.30 (-)
C(6')	29.20	29.20	29.20 (29.1)	29.30 (-)
C(7')	29.20	29.20	29.20 (29.1)	29.30 (-)
C(8')	29.20	29.20	29.20 (29.1)	29.30 (-)
C(9')	24.37	24.21	25.62 (25.8)	26.39 (-)
C(10')	43.50	43.46	43.56 (43.8)	43.62 (-)
C(11')	208.86	208.82	208.92 (209.3)	209.10 (-)
C(12')	23.72	23.61	23.71 (23.6)	23.78 (-)
COO(3)	165.65	170.14	-	-
CH <sub>2</sub> COO(3)	-	* 21.85	-	-
CH aromatic	132.83	-	-	-
CH aromatic	130.44	-	-	-
CH aromatic	129.37	-	-	-
CH aromatic	128.29	-	-	-

\* may be further corrected.

( ) from literature of cassine(145), iso-6-cassine(144)

2) Polar part, which was postulated to be sodium benzoate, was reversed to acid and extract. This part was identified to be benzoic acid from following datas

HPLC of Unknown benzoic acid : (figure 12)

retention time = 5.68 min.  
(standard = 5.69 min.)

Chromatographic Condition

Column : Phenomenex packed with  $\mu$  Bondapack C<sub>18</sub>,  
10 $\mu$ , 300 x 3.9 mm.  
Mobile phase : Methanol : 1% Acetic acid in water  
50 : 50  
Flow rate : 1.0 ml/min  
Detector : UV 254 nm, AUFS 1.0  
Injection Volume : 20  $\mu$ l

MS of Unknown benzoic acid : m/e (%rel) = 122(M<sup>+</sup>)(82.77),  
(figure 13) 105(100.0), 77(65.70).

<sup>1</sup>H-NMR of Unknown benzoic acid :  $\delta$  = 7.51(m, 3H), 8.12(dd, 2H).  
(figure 14)

The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of L-1 show that structure of L-1 would be 2,6-disubstituted piperidine. Methyl group that is substituted at position 2 shows a singlet(1.19 ppm), which was confirmed by irradiation of proton at position 2 (3.39 ppm); resulting a higher intensity singlet signal at 1.2 ppm (figure 7b).

2-Dodecanone side chain at position 6 was obtained from these datas : <sup>1</sup>H-NMR;  $\delta$  = 1.27(s, 18H), 2.12(s, 3H), 2.42

(t, 2H) ppm (15-18) and  $^{13}\text{C}$ -NMR;  $\delta$ =23.72, 24.37, 29.20, 34.56, 43.50, 208.86 ppm (143-145)

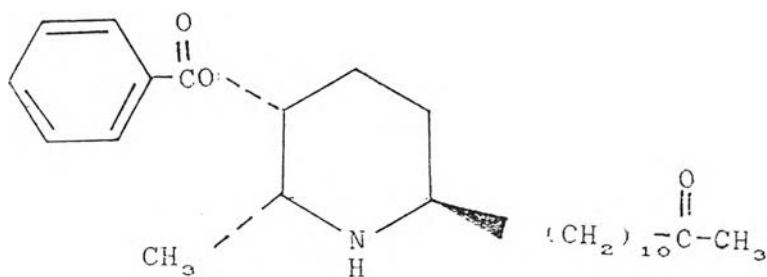
Accordingly informations of infrared spectrum ( $\nu_{\text{max}}$ ) = 1,600, 1,580, 1,520  $\text{cm}^{-1}$ , proton-NMR spectrum ( $\delta$ =7.49 (3H), 8.06 (2H) ppm) and carbon-13 NMR spectrum ( $\delta$ =132.83, 130.44, 129.37 and 128.29 ppm), it is indicated that L-1 contains aromatic portion (146, 147) in its structure which might be substituted at position 3 of piperidine ring. Chemical shift of proton at position 3 ( $\delta$ =5.07 ppm), when was compared with those of cassine ( $\delta$ =3.54 ppm) and iso-6-cassine ( $\delta$ =3.54 ppm) (15, 16, 17), was moved to downfield ( $\delta$ =5.08 ppm) due to anisotropic and resonance effects of carbonyl group and aromatic part which was substituted in this position.

$^{13}\text{C}$ -NMR of L-1 was shown carbonylic carbon of ester at  $\delta$ =165.65 ppm (148) and infrared spectrum at 1,720 and 1,280  $\text{cm}^{-1}$  were bands of ester when compared with methyl benzoate that showed absorption bands at 1,740 and 1,205  $\text{cm}^{-1}$  (146). Hence it was suggested that aromatic ring was substituted at position 3 instead of hydroxyl group; with ester linkage.

Mass spectrum of L-1 showed molecular ion peak ( $M^+$ )=401, respectively to this structure of 2-methyl-3-benzoyl-6-(11'-oxododecyl)-piperidine ( $\text{C}_{25}\text{H}_{39}\text{NO}_3$ ). The fragmentations were formed by loss of  $-\text{CH}_3$  ( $m/e$  = 386,  $M^+$ -15), loss of  $-\text{CH}_2-\text{CO}-\text{CH}_3$  ( $m/e$ =344,  $M^+$ -57) which is a typical fragment unit of 2-dodecanone side chain (15), and loss of benzoyl moiety ( $m/e$ =280,  $M^+$ -121) or benzoic acid moiety ( $m/e$ =279,  $M^+$ -122).

Hydrolysis product D-1 was identified by IR, MS,  $^1\text{H-NMR}$  to be iso-6-cassine by comparison with authentic spectra (figure 31 b. and figure 33 b.) and literature (16,17). Unknown benzoic acid was identified by high performance liquid chromatography (figure 12) and UV spectrometry (figure 13) to be benzoic acid. Mass spectrum of this unknown benzoic acid (figure 14) showed large molecular ion peak ( $m/e=122$ ) and the characteristic fragmentation of benzoic acid (146) by loss of  $-\text{OH}$  ( $m/e=105$ ,  $M^+-17$ ) and loss of  $-\text{COOH}$  ( $m/e=77$ ,  $M^+-45$ ). Proton NMR of this unknown benzoic acid would also show aromatic ring of benzoic acid at  $\delta=7.51(3\text{H})$  and  $8.12(2\text{H})$  ppm.

Therefore all these informations suggest L-1 should be 2-methyl-3-benzoyloxy -6-(11'-oxododecyl)piperidine, ( $2\alpha, 3\alpha, 6\beta$ ). Structure of L-1 (99) is shown below.



(99)

L-2 : This compound (200 mg, 3.8 mg%) was pale yellow clear oil. L-2 was very miscible with chloroform and methanol.

M.W. : 339 (EIMS)

TLC : Rf values and results of detection were summarized as follow

Rf value	0.83	in solvent system	A
	0.89	in solvent system	B
	0.89	in solvent system	C
	0.85	in solvent system	D
	0.89	in solvent system	E

- i fluorescent at UV 254 nm.
- ii brown spot (positive result) with iodine vapour.
- iii orange spot (positive alkaloidal result) with Dragendorff's reagent.
- iiii quenching (secondary amine) with fluoescarmine

UV :  $\lambda_{\max}$  (in  $\text{CHCl}_3$ ) = 242, 280 nm.  
: (figure 16)

IR : (figure 17)

$\lambda_{\max}$ ( $\text{cm}^{-1}$ )	=	3,400-3,300	N-H stretching
		2,950-2,850	C-H stretching of $\text{CH}_3, \text{CH}_2$
		1,740	C=O stretching of ester
		1,720	C=O stretching of ketone
		1,660-1,670	N-H stretching
		1,430-1,470	C-H bending (asymmetric)
		1,365	C-H bending (symmetric)
		1,240	C-O stretching of ester



MS : (figure 18)

m/e (% relative) = 340(M<sup>+</sup>+1)(0.47), 339(M<sup>+</sup>)(1.47),  
 324(1.49), 296(1.31), 282(6.33),  
 279(6.41), 264(2.65), 236(1.66),  
 222(6.56), 166(1.25), 156(100),  
 154(3.81), 138(2.68), 124(5.01),  
 110(7.43).

<sup>1</sup>H-NMR : (figure 19)

<u>chemical shift</u>	<u>multiplicity</u>	<u>No. of proton (remark)</u>
1.14	doublet	3H (CH <sub>3</sub> at C-2)
1.27	singlet	18H (CH <sub>2</sub> of side chain)
2.07	singlet	3H (CH <sub>3</sub> adjacent to COO)
2.13	singlet	3H (CH <sub>3</sub> adjacent to C=O)
2.42	triplet	2H (CH <sub>2</sub> adjacent to C=O)
2.80	multiplet	1H (H at C-6)
3.26	multiplet	1H (H at C-2)
4.82	multiplet	1H (H at C-3)

<sup>13</sup>C-NMR : (figure 20, table 4;page 47)

Confirmation of structure and stereochemistry of L-2 by acetylation of compound with known conformation; cassine and iso-6-cassine, spectral datas of acetylated products are summerized as following.

IR of AC-1 :  $\nu_{\max}$  = 1,015, 1,160, 1,240, 1,365, 1,420,  
 (figure 21) 1,470, 1,640, 1,720, 1,740, 2,850,  
 2,950, 3,500 cm<sup>-1</sup>.

IR of AC-2 :  $\nu_{\max}$  = 1,015, 1,160, 1,240, 1,365, 1,410,  
 (figure 22) 1,470, 1,645, 1,720, 1,740, 2,850,  
 2,950, 3,500 cm<sup>-1</sup>.

$^1\text{H-NMR}$  of AC-1 :  $\delta = 1.20(\text{d}^*, 3\text{H}), 1.26(\text{s}, 18\text{H}), 2.06(\text{s}, 3\text{H}),$   
 (figure 23)  $2.12(\text{s}, 3\text{H}), 2.13(\text{s}, 3\text{H}), 2.42(\text{t}, 2\text{H}),$   
 $3.71(\text{m}, 1\text{H}), 4.23(\text{sp}, 1\text{H}), 4.6-5.0(\text{m}, 2\text{H}).$

$^1\text{H-NMR}$  of AC-2 :  $\delta = 1.22(\text{d}^*, 3\text{H}), 1.27(\text{s}, 18\text{H}), 2.07(\text{s}, 3\text{H}),$   
 (figure 24)  $2.12(\text{s}, 3\text{H}), 2.13(\text{s}, 3\text{H}), 2.42(\text{t}, 2\text{H}),$   
 $3.8-4.8(\text{broad s}, 2\text{H}), 5.12(\text{sx}, 1\text{H}).$

(\* might be corrected)

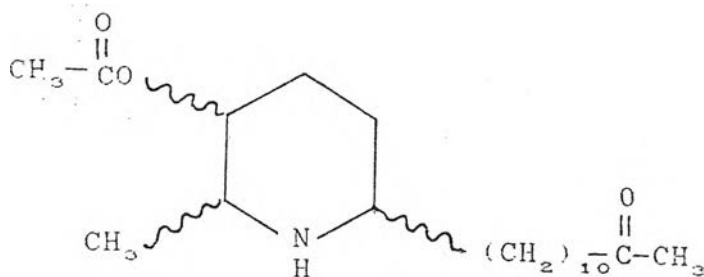
Structure of L-2 was identified to be a piperidine ring with methyl group at position 2 ( $^1\text{H-NMR}; \delta = 1.14$  ppm, 3H) and 2-dodecanone side chain at position 6 ( $^1\text{H-NMR}; \delta = 1.27$  (18H), 2.13 (3H) and 2.42 (2H) ppm when compared with known compounds (15-18).

The infrared spectrum of L-2 indicated strong absorption band of ester at  $\nu_{\text{max}} = 1,740$  and  $1,240 \text{ cm}^{-1}$  (146) while the carbon-13 spectrum showed carbonylic carbon of ester at  $\delta = 170.14$  ppm (148). The  $^1\text{H-NMR}$  signal at  $\delta = 2.07$  ppm. and carbon-13 NMR signal at  $\delta = 21.85$  ppm reveal the presence of methyl group that adjacent to carbonyl of ester, so there are acetate portion in the structure of L-2. Chemical shift of proton at position 3 was moved to downfield ( $\delta = 4.82$  ppm), suggests that acetate group is substituted at position 3.

The mass spectrum of L-2 was shown molecular ion peak ( $\text{M}^+$ ),  $m/e = 339$ , representatively to this proposed structure and the fragmentations of L-2 was shown by loss of  $-\text{CH}_3$  ( $m/e = 324, \text{M}^+ - 15$ ), loss of  $-\text{CH}_2-\text{CO}-\text{CH}_3$  ( $m/e = 282, \text{M}^+ - 57$ ) loss of  $-\text{COO}-\text{CH}_3$  ( $m/e = 280, \text{M}^+ - 59$ ) and loss of  $\text{CH}_3\text{COOH}$  ( $m/e = 279, \text{M}^+ - 60$ ).

By evidences of IR and  $^1\text{H-NMR}$  of Ac-1 and Ac-2 which were showed that Ac-1 and Ac-2 were diacetylated products of cassine and iso-6-cassine ; O-acetylation of Ac-1:  $\nu_{\text{max}} = 1,740$  and  $1,240 \text{ cm}^{-1}$ ;  $\delta = 2.06$  (s, 3H) ppm, Ac-2:  $\nu_{\text{max}} = 1,740$  and  $1,240 \text{ cm}^{-1}$ ;  $\delta = 2.07$  (s, 3H) ppm. and N-acetylation of Ac-1:  $\nu_{\text{max}} = 1,640 \text{ cm}^{-1}$ ;  $\delta = 2.12$  (s, 3H) ppm , Ac-2:  $\nu_{\text{max}} = 1,645 \text{ cm}^{-1}$ ;  $\delta = 2.10$  (s, 3H) ppm, it could be summarized L-2 to be acetyl derivative of cassine or iso-6-cassine that is substituted at position 3, respectively.

Therefore structure of L-2 is 2-methyl-3-acetyl-6-(11-oxododecyl)piperidine, which is shown below (100)



(100)

L-3 : This compounds (300 mg, 5.8 mg%) were white needle crystals. L-3 was freely soluble in chloroform and methanol, slightly soluble in hexane and insoluble in water.

M.W. : 297 (EIMS)

M.P. : 71-72 °C

TLC : Rf values and result of detection were summerized as follow

Rf value	0.60	in solvent system	A
	0.70	in solvent system	B
	0.55	in solvent system	C
	0.55	in solvent system	D
	0.69	in solvent system	E

- i fluorescent at UV 254 nm.
- ii brown spot (positive result) with iodine vapour.
- iii orange spot (positive alkaloidal result) with Dragendorff's reagent.
- iiii quenching (secondary amine) with fluoescarmine

UV :  $\lambda_{\max}$  (in  $\text{CHCl}_3$ ) = 242 nm  
: (figure 25)

IR : (figure 26 a.)

$\nu_{\max}$ ( $\text{cm}^{-1}$ )	= 3,400-3,300	O-H, N-H streching
	2,900-2,850	C-H streching of $\text{CH}_3$ , $\text{CH}_2$
	1,705	C=O streching of ketone
	1,415-1,470	C-H bending (asymmetric)
	1,360	C-H bending (symmetric)

MS : (figure 27), EIMS.

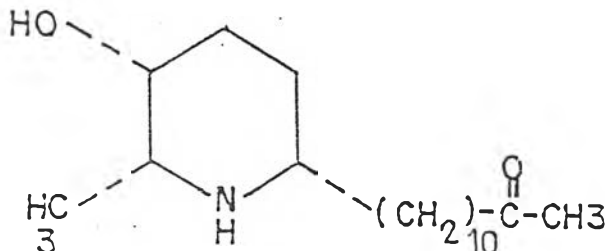
m/e (% relative) = 298(M<sup>+</sup> +1)(0.45), 297(M<sup>+</sup>)(1.66),  
282(1.54), 254(0.64), 240(7.85),  
238(1.79), 222(0.86), 212(0.74),  
156(0.58), 114(100.0), 96(9.68).

<sup>1</sup>H-NMR : (figure 28 a.)

<u>chemical shift</u>	<u>multiplicity</u>	<u>No. of proton</u> (Remark)
1.08	doublet	3H (CH <sub>3</sub> at C-2)
1.27	singlet	18H (CH <sub>2</sub> of side chain)
2.13	singlet	3H (CH <sub>3</sub> adjacent to C=O)
2.42	triplet	2H (CH <sub>2</sub> adjacent to C=O)
2.74	multiplet	1H, 1H (H at C-2, C-6)
3.54	singlet	1H (H at C-3)

<sup>13</sup>C-NMR : (figure 29, table 4;page 47)

L-3 was identified by Rf values on TLC along with the authentic sample (cassine) and all spectral datas, compared with those of the known compound in the literature (15,17,145) and of the authentic sample (cassine), to be cassine (101).



(101) Cassine

The infrared spectrum of L-3 was identical to that of the authentic cassine (figure 26 b.) and was shown the presences of carbonyl, hydroxyl and secondary amine groups.

The molecular ion peak and the fragmentations in the mass spectrum of L-3;  $m/e = 297 (M^+)$ ,  $282 (M^+ - CH_3)$ ,  $240 (M^+ - 57)$ , specific loss of  $-CH_2-CO-CH_3$  moiety of this 2-dodecanone side chain),  $114$  (base peak), were very similar to those of cassine (15, 17).

The  $^1H$ -NMR spectrum of L-3 was identical to that of the authentic cassine (figure 28 b). The signal of carbinol proton at position 3 ( $\delta = 3.54$  ppm, singlet) with the width at half height ( $\omega_{1/2} = 6$  Hz) was known to be characteristic of an equatorial proton in the 3-piperidinol derivatives (149).

The  $^{13}C$ -NMR spectrum of L-3 was also similar to that of cassine (145). The higher chemical shift of carbon at position 2 and 6 of piperidine ring provided evidence for *cis*-2,6-dialkyl piperidine (150).

L-4 : This compound (150 mg, 2.9 mg%) was clear-colourless oil. L-4 was very miscible with chloroform and methanol.

M.W. : 297 (EIMS)

TLC : Rf values and results of detection were summarized as follow

Rf value	0.42	in solvent system	A
	0.44	in solvent system	B
	0.33	in solvent system	C
	0.36	in solvent system	D
	0.52	in solvent system	E

- i fluorescent at UV 254 nm.
- ii brown spot (positive result) with iodine vapour.
- iii orange spot (positive alkaloidal result) with Dragendorff's reagent.
- iiii quenching (secondary amine) with fluorescamine

UV :  $\lambda_{\max}$  (in  $\text{CHCl}_3$ ) = 242 nm  
: (figure 30)

IR : (figure 31a.)

$\lambda_{\max}$ ( $\text{CM}^{-1}$ ) = 3,400	O-H, N-H stretching
2,950, 2890	C-H stretching
1,715	C=O stretching of ketone
1,430-1,460	C-H bending (asymmetric)
1,360	C-H bending (symmetric)

MS : (figure 32)

m/e(%relative) = 298(M<sup>+</sup>+1)(0.88), 297(M<sup>+</sup>)(1.90), 282  
(1.38), 240(5.92), 238(1.58), 212  
(0.84), 114(100.0), 96(8.3).

<sup>1</sup>H-NMR : (figure 33 a.)

<u>chemical shift</u>	<u>multiplicity</u>	<u>No. of proton (remark)</u>
1.12	doublet	3H (CH <sub>3</sub> at C-2)
1.26	singlet	18H (CH <sub>2</sub> of side chain)
2.13	singlet	3H (CH <sub>3</sub> adjacent C=O)
2.42	triplet	2H (CH <sub>2</sub> adjacent C=O)
3.08	multiplet	1H (H at C-6)
3.14	multiplet	1H (H at C-2)
3.68	singlet	1H (H at C-3)

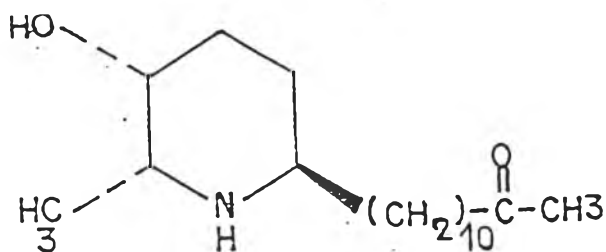
<sup>13</sup>C-NMR : (figure 34, table 4;page 47)

The R<sub>f</sub> values on TLC of L-4 gave the same values of the authentic iso-6-cassine. The mass spectrum of L-4 was similar to those of cassine(15) and iso-6-cassine (16, 17), while the IR and <sup>1</sup>H-NMR spectra of L-4 were very similar to those of authentic iso-6-cassine (figure 31b. and 33b.). The width at half height ( $\omega_{1/2}=18 \text{ Hz}$ ) of L-4 very broad signal of carbinol proton at position 3 suggested the axial carbinol proton(149).

The <sup>13</sup>C-NMR of L-4, as well other spectral datas, was similar to that of iso-6-cassine (144). The low chemical shifts of carbon in piperidine ring indicated that structure of L-4 was *trans*-2,6-dialkyl piperidine (150).



According to mostly informations obtained from L-4, it was able to identify L-4 to be iso-6-cassine (102)



(102) Iso-6-cassine

L-5 : This compound (80 mg, 1.5 mg%) was pale yellow oil. L-5 was miscible with chloroform and methanol.

M.W. : 325 (EIMS)

TLC : Rf values and results of detection were summarized as follow

Rf value	0.57	in solvent system	A
	0.79	in solvent system	B
	0.63	in solvent system	C
	0.62	in solvent system	D
	0.75	in solvent system	E

- i fluorescent at UV 254 nm.
- ii brown spot (positive result) with iodine vapour.
- iii orange spot (positive alkaloidal result) with Dragendorff's reagent.
- iiii quenching (secondary amine) with fluorescamine

UV :  $\lambda_{\max}$  (in  $\text{CHCl}_3$ ) = 242 nm  
: (figure 35)

IR : (figure 36)

$\lambda_{\max}$ (cm <sup>-1</sup> ) =	3,400-3,300	O-H, N-H stretching
	2,950, 2,850	C-H stretching of $\text{CH}_3, \text{CH}_2$
	1,710	C=O stretching of ketone
	1,415-1,470	C-H bending (asymmetric)
	1,370	C-H bending (symmetric)

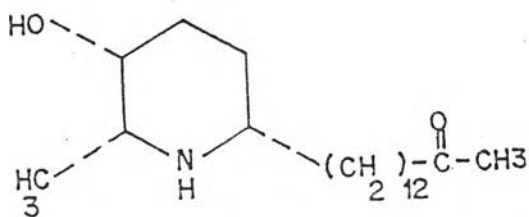
MS : (figure 37)

m/e(%relative) = 326( $M^+ + 1$ ) (0.02), 325( $M^+$ ) (0.12), 310(0.11),  
282(1.48), 268(0.92), 240(6.84), 212(0.75),  
149(3.25), 114(100.0), 96(8.76).

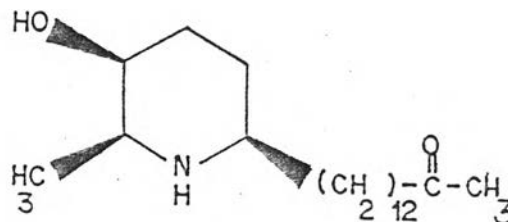
$^1\text{H-NMR}$  : (figure 38)

chemical shift	multiplicity	No. of proton (remark)
1.10	doublet	3H ( $\text{CH}_3$ at C-2)
1.26	singlet	2H ( $\text{CH}_2$ of side chain)
2.13	singlet	3H ( $\text{CH}_3$ adjacent to C=O)
2.42	triplet	2H ( $\text{CH}_2$ adjacent to C=O)
2.76	multiplet	1H (H at C-6)
2.76	multiplet	1H (H at C-2)
3.56	singlet	1H (H at C-3)

L-5 had IR spectrum quite similar to cassine, suggested the structure of L-5 may similar to cassine. The Mass spectrum of L-5 showed molecular ion peak = 325, corresponding to molecular formula  $\text{C}_{20}\text{H}_{39}\text{NO}_2$ , with the fragmentations  $m/e = 310(M^+ - 15)$ ,  $268(M^+ - 57)$  and = 114 (base peak) indicated two further methylene groups were extended from 2-dodecanone side chain of cassine or iso-6-cassine. Therefore L-5 could be cassinicine (103) (15) or spectaline (104) (16). Structures are shown below.



(103) Cassinicine



(104) Spectaline

## Discussion

Stereochemistry of compound L-2 and L-5 are not confirmed, for these following reasons, it was suggested L-2 to be 3-acetyl derivative of iso-6-cassine

1. Cassine is more stable to be acetylated than iso-6-cassine, because of intramolecular hydrogen-bonding between hydroxyl group at position 3 and the N atom of piperidine nucleus of cassine is more favorable than iso-6-cassine (16).

2. Carbon-13 spectrum of L-2 is quite similar to that of iso-6-cassine, especially chemical shifts of carbon atoms of piperidine nucleus.

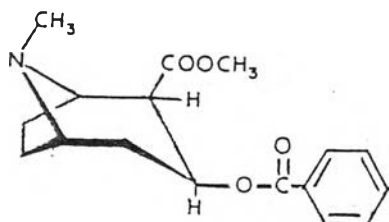
More experiments should be needed to confirm the stereochemistry of L-2, and of L-5 with high resolution spectroscopic methods.

Some biological activities that alkaloids from the flowers of *Cassia spectabilis* DC. could be expected to possess and concern, are local anesthetic, cholinergic, and antimalarial activities.

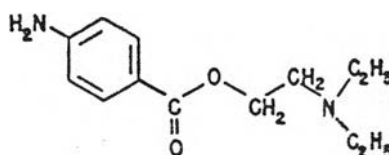
### 1. Local Anesthetic Agents

Cocaine(105) is an alkaloid from the leaves of *Erythroxylon coca* and other species of *Erythroxylon* (*Erythroxylaceae*), that has local anesthetic property. Cocaine is a prototype to develop other synthetic local anesthetic agents, especially benzoic acid derivatives such as procaine(106) which molecules must be contained of aromatic portion (A)-intermediate chain (B)-amine portion

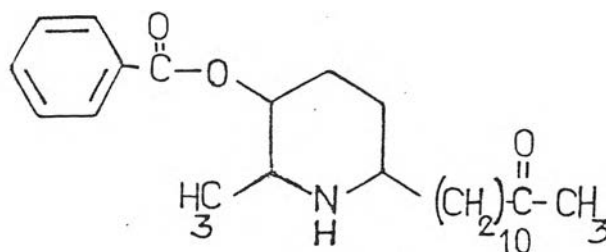
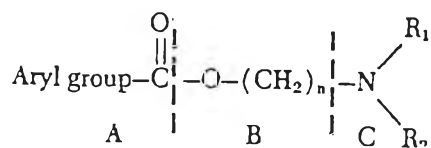
(C) (151).



(105) Cocaine



(106) Procaine

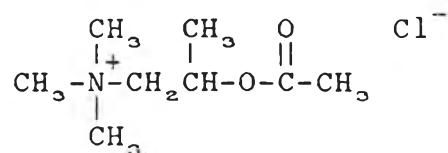
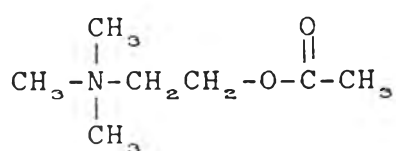


(107) L-1

Alkaloid L-1 exists the required structure of ester of benzoic acid local anesthetic derivatives, as benzoic acid (A)-(CH<sub>2</sub>-CH<sub>2</sub>-)(B)-secondary amino groups of piperidine ring (C).

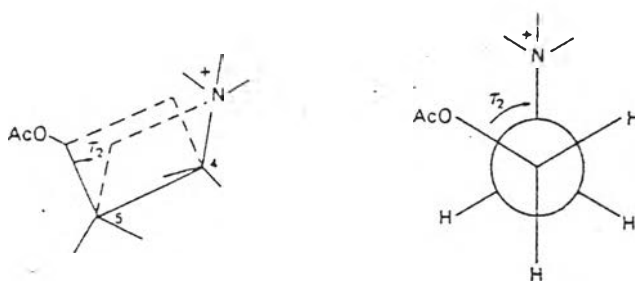
## 2. Cholinergic Agents

Acetylcholine (108) and related compounds; cholinergic agonists such as metacholine chloride (109) require structure which has ester group and trimethylammonium groups ( $R-N^+(CH_3)_3$ ;  $R = -C-C-$ ). Stereochemistry of compounds containing a choline component (i.e.,  $O-C-C-N^+(CH_3)_3$ ) have a preferred synclinal (gauche) conformation with the torsion angle ( $\tau_2$ ) values ranging from  $68-89^\circ$ .

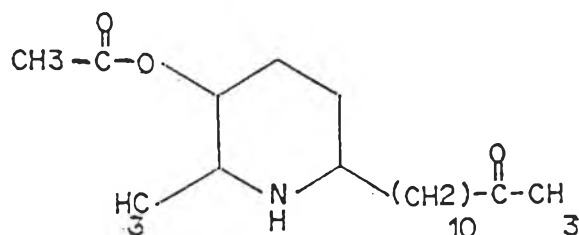


(108) Acetylcholine

(109) Metacholine Chloride



Gauche conformation of Acetylcholine



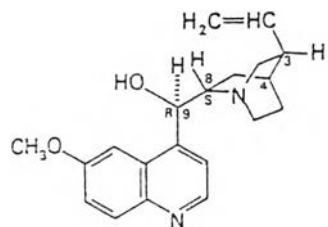
L-2 [2-methyl-3-acetyl-6-(11-oxododecyl)-piperidine]

L-2 has acetyl ester group, secondary amine group.

in its structure, if L-2 is derivatized to quaternary ammonium group it might provide some cholinergic activity.

### 3. Antimalarials

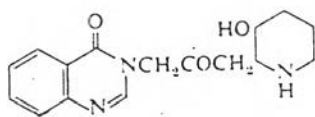
Quinine (110) and other Cinchona alkaloids contain piperidine ring in their quinuclidine rings (111). Alkaloid from *Dichroa febrifuga*; febrifugine (112), as well as synthetic 4-quinolinemethanol; mefloquine (113) also have piperidine ring as their side chains.



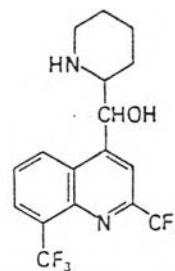
(110) Quinine



(111) Quinuclidine



(112) Febrifugine



(113) Mefloquine

All isolated alkaloids from the flowers of *Cassia spectabilis* DC. in this research have piperidine nucleus in their structures and might be concerned with antimalarial property.

### Conclusion

There are about 30 families, 140 species in plant kingdom that are reported to contain piperidine alkaloids. (39-68, 88-141).

In this research, five piperidine alkaloids were isolated by mean of chromatography and identified their structures on the basis of chemical and spectroscopic methods.

L-1 was identified to be 2-methyl-3-benzoyloxy-6-(11'-oxododecyl)piperidine, (2 $\alpha$ ,3 $\alpha$ ,6 $\beta$ ) and L-2 to be 2-methyl-3-acetyl-6-(11'-oxododecyl)piperidine. These two compounds are piperidine alkaloids which have never been reported to be found in this plant of *Cassia spectabilis* DC., previously. L-3 was cassine and L-4 was iso-6-cassine, while L-5 was spectaline or cassinicine (15-18).