

Chapter IV

Discussion

There is no previous report on the chemical constituents of the leaves of *Litsea cubeba*. In this present work, the isolation of two isoquinoline alkaloids of the aporphine type was carried out. The structures of these compounds were elucidated by spectroscopic methods and comparison with reported values.

1. Structure elucidation of compound Z-35

Compound Z-35 is obtained as amorphous, green mass (43 mg yield 0.0005 %).

The ei mass spectrum of compound Z-35 (Figure 3 , page 124) exhibits molecular ion peak at m/z 325 (93.0 %) , thus establishes molecular formula as $C_{19}H_{19}O_4N$. The base peak 324 $(M-1)^+$ and the uv absorption spectrum at 310 and 290 nm (Figure 4 , page 125) suggesting its aporphine nature with substitutions at 1,2,9 and 10 (Shamma, 1972). A broad absorption band at 3400 cm^{-1} in its ir spectrum (Figure 4 , page 126) indicates the presence of one or more hydroxyl group in the structure. Another band at 1030 cm^{-1} suggests the presence of methylenedioxy function.

The relative stereochemistry of this compound was determined. It exhibits the right-handed optical rotation property of $[\alpha]^{25}_D -512^\circ$ (c 1.4 mg/ml, MeOH),

Its ^1H nmr spectrum exhibits one methoxy singlet at δ 3.91 ppm and three aromatic singlets at δ 7.93, 6.75 and 6.55 ppm , and an N-Me (δ 2.58 ppm). The characteristic singlet signals of H-11 (δ 7.93 ppm), 2-OMe (δ 3.91 ppm) and the

multiplicity of the aromatic protons support the substitution pattern suggested earlier by the eims and uv spectra of the compound.

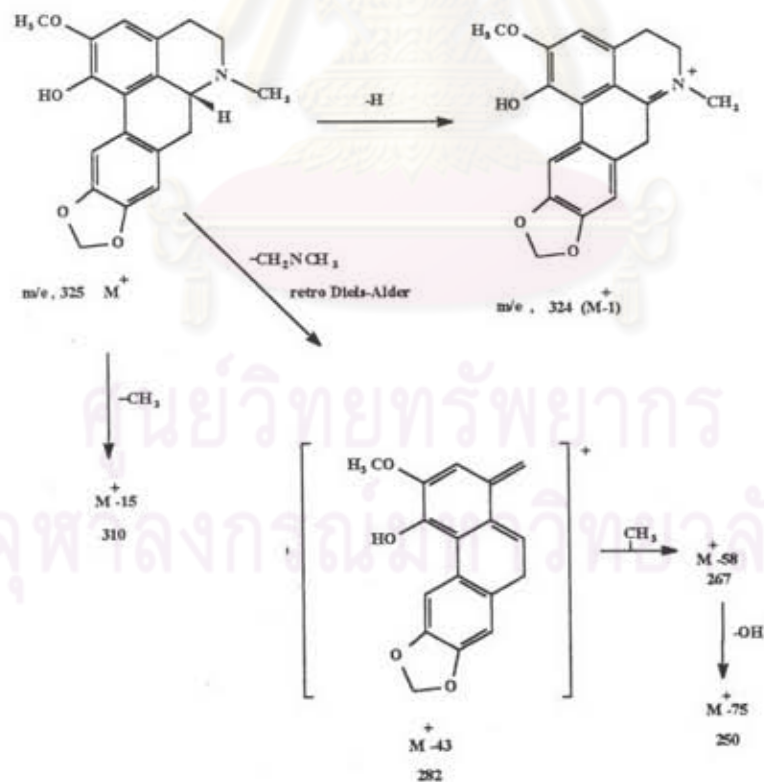
As C-2 is already substituted with a methoxyl group, the methylenedioxy function should therefore be located at C-9,10. The remaining position for phenolic hydroxyl group to be placed must be at C-1. These deductions lead to the identification of compound Z-35 as (-)-domesticine (Johns *et al.*, 1966). The complete ^1H and ^{13}C nmr assignments of compound Z-35 were obtained by the analysis of proton and carbon chemical shifts and their correlations observed in 2D-nmr spectra.

As evidenced by cross peaks observed in the ^1H - ^1H COSY spectrum (Figure 9, page 130) and its splitting pattern, the triplet signal at δ 4.18 ppm, which couples to the multiplet signals at δ 2.19 and 2.32 ppm, can be assigned to the proton at 6a. The latter two signals would then be assigned to the two protons of C-7. The signal at δ 2.64 and 3.00 ppm were assigned to methylene protons at position 4 according to their long-range coupling with C-3 signal at δ 108.3 ppm. The multiplet signal at δ 3.15 ppm (integrated for two protons), which shows correlation with the H-4 methylene proton in ^1H - ^1H COSY spectrum, would then be readily assignable to the two methylene protons of position 5.

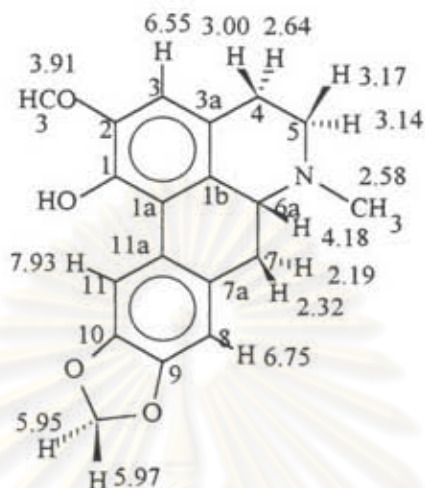
Based on the study of an aporphine alkaloid which has correlated structure, (+)-glaucine, Kerr and co-workers (1986) have suggested that, regardless of the conformation of the molecule, the α -protons of this group of alkaloids are deshielded relative to the β -protons. Hence, initial proton assignments of (-)-domesticine could be made for the H-5 β (δ 3.17 ppm), H-5 α (δ 3.14 ppm), H-4 β (δ 3.00 ppm), H-4 α (δ 2.64 ppm), H-7 β (δ 2.32 ppm) and H-7 α (δ 2.19 ppm).

The assignments of the carbon signals of compound Z-35, base on their chemical shifts and 2D nmr data, are also carried out (Table 6). The proton-attached carbon signals were assigned directly from a ^1H - ^{13}C COSY spectrum (Figure 10 , page 131). The signals of quaternary carbons: C-1 (δ 140.8 ppm), C-2 (δ 146.1 ppm), C-3 (δ 108.3 ppm), C-10 (δ 146.3 ppm), C-11a (δ 125.7 ppm) and C-1b (δ 62.3 ppm) were assigned by a COLOC spectrum (Figure 11 , page 132) from their long range couplings to the proton signals of H-3 (δ 6.55 ppm), 2-OMe (δ 3.91ppm), H-4 (δ 2.64 ppm), H-8 (δ 6.75 ppm) and N-Me (δ 2.58 ppm).

The proposed structure of compound Z-35 was also supported by the analysis of mass fragmentations of its eims. Proposed fragmentation pattern of compound Z-35 is as follows:



Chemical structure of compound Z-35 is shown below.



The assignments for all protons and carbons nmr signals, as well as the long-range correlations observed in the COLOC spectrum are summarized in table 6.

Comparison to the ^{13}C nmr signal assignment previously reported in the literature (Kano *et al.*, 1976), there were some point difference. The position of 4-C (δ 34.6 ppm) and 7-C (δ 27.7 ppm) were interchanged, as a consequence of recent observed from more efficiency and modern spectroscopic experiments.

The proton assignment of domesticine was in agreement with the previous reported of the alkaloid by Johns and co-workers (1966). The full proton assignment of the alkaloid is reported here for the first time.

Domesticine have been reported to be found in the family Berberidaceae (*Nantida*)(Guinadeau *et al.*, 1975), Fumaridaceae (*Corydalis*) (Guinadeau *et al.*, 1983), Papaveraceae (*Corydalis*, *Glaucium*) (Naruto and Kaneko, 1972). In the family Lauraceae it was found only in the genus *Cassytha* (Johns *et al.*, 1966b). Domesticine has

never been reported as constituent of this plant species and this is the first report of its occurrence in the genus *Litsea*.

Table 6 ^{13}C and ^1H signal assignments of compound Z-35 and long-range correlation between carbons and protons.

Position	C(δ ppm)	H(δ ppm)	long-range correlation from C on H observed in COLOC spectrum
1	140.8	-	H-3
1a	119.5*	-	-
1b	62.3	-	-
2	145.9	-	2-OCH ₃
3	108.3	6.55(1H, s)	H-4
3a	123.5*	-	-
4 α	34.6	2.64 (1H, dd, $J=4, 14.1, 14.1$ Hz)	-
4 β	-	3.00 (1H, dd, $J=4.0, 14.1$ Hz)	-
5 α	53.1	3.14 (1H, dd, $J=5.5, 10.7$ Hz)	-
5 β	-	3.17 (1H, ddd, $J=1.2, 4.0, 13.3$ Hz)	-
6a	62.2	4.18(1H, dd, $J=8,8$)	-
7 α	27.7	2.19(1H, m)	-
7 β	-	2.32(1H, m)	-

Table 6 cont.

Position	C (δ ppm)	H (δ ppm)	long-range correlation from C on H observed in COLOC spectrum
7a	130.0*	-	-
8	108.8	6.75(1H, s)	-
9	146.1	-	-
10	146.3	-	H-8
11	109.1	7.93(1H, s)	-
11a	125.7	-	H-8
-OCH ₂ O-	100.8	5.97(1H, d, $J=1.5$) 5.95(1H, d, $J=1.5$)	-
-NCH ₃	43.3	2.58(3H, s)	-
2-OCH ₃	56.2	3.91(3H, s)	-

*may be interchanged

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Structure elucidation of compound G-47

Compound G-47 was obtained as pale pink plates from methanol, yield 136 mg. (0.026 %).

The eims spectrum of the compound (Figure 12 , page 133) shows the molecular ion peak at m/z 327 (90.6%) which correspond to the molecular formula of $C_{19}H_{21}NO_4$. The base peak from eims, 326 $[M-1]^+$ the uv absorption at λ max 241.4 and 310 nm (Figure 13, page 134) display the characteristic of aporphine substituted at 1, 2, 9 and 10 (Shamma, 1972).

The ir spectrum (Figure 14 , page 135) shows absorption typical of hydroxyl group at $3200-3500\text{ cm}^{-1}$.

The ^1H nmr spectrum (Figure 15 , page 136) exhibits two methoxy proton signal at δ 3.91 and δ 3.98 ppm, an N-methyl protons δ 2.53 ppm and three aromatic singlets at δ 8.01, 6.82 and 6.54 ppm. These deductions and its right-handed optical property ($[\alpha]^{25} +11^\circ, C$ 13.2 mg/ml, MeOH), suggest that this compound is (+)-isoboldine (Johns *et al.*, 1966).

Assignments of all proton signals were aided by both one and two-dimensional homonuclear, decoupling experiments and 2-D nmr spectra.

The singlet signals at δ 6.54, 6.82 and 8.01 ppm could be assigned as those of H-3, H-8 and H-11, respectively. Two methoxy singlet signal at δ 3.91 and 3.93 ppm were assigned as 2-OMe and 10-OMe.

The proton signals at δ 2.3-3.3 ppm (Figure 20 , page 141) consist of the alicyclic and N-methyl peaks of isoboldine. Integration of this region showed that the multiplets at δ 3.14, 2.98, 2.63, 2.55, 2.53 and 2.51 ppm each represent one proton, while the multiplet centered at δ 3.02 ppm was integrated for two protons.

The seven alicyclic protons of isoboldine showed up as two isolate spin systems, one being the 6a and 7-methylene protons. These two systems can be identified by the inspection of 1D decoupling experiments (Figure 21 , page 143), in which irradiation of the signal at δ 3.14 ppm caused the collapse of the signal at δ 2.64 ppm to a broad singlet with some simplification of the signals at δ 3.02 and 2.51 ppm while no change was observed in the δ 3.04, 2.98 ppm signals and 2.55 ppm while another irradiation of the δ 2.98 ppm signal caused the collapse of the signal at δ 2.55 ppm.

In addition, confirmation of the assignments of the two ring systems of isoboldine was made by a 2D homonuclear experiment. The coupling interactions (cross peaks) among the signals at δ 2.98, 2.55 and 3.04 ppm and the correlation between the signals at δ 6.82 ppm (H-8) and 2.55 ppm (H-7) suggest that the 6a and 7-methylene protons fall into these region. While the correlation of the signal at δ 6.53 ppm (H-3) to these at δ 3.14 and 2.64 ppm suggested the latter the proton signals of 4-methylene position. The remaining signals (δ 2.51 and 3.02 ppm) can then be readily assigned as two proton signals of 5-methylene position.

The assignments for the four methylene protons at positions 4 and 5 can be resolved according to Kerr and co-workers (1986), Glaser and co-workers (1991), and from the splitting pattern and the large vicinal coupling constant (12.7 Hz) observed for the ^1H -nmr signals at δ 3.14 ppm (H-4) and 2.51 ppm (H-5) suggest that these proton

signals belong to H-4 α and H-5 β , while the doublet of doublet signals (dihedral angle =90°) at δ 2.63 (H-4) and 3.02 (H-5) ppm would belong to H-4 β and H-5 α , respectively.

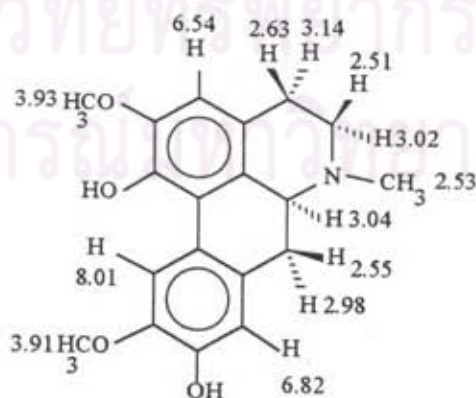
The diastereotopic H-7 α , H-7 β protons have different relationship to H6a. For the larger coupling constant (δ 4.27 Hz), dihedral angle about 40°, which means that the signal at δ 2.98 ppm should be that of H-7 α and the signal at δ 2.55 ppm with smaller J value of 0.92 Hz, resulting from dihedral angle about 110°, would be the H-7 β signal.

The carbon signals were assigned directly from the correlation peak observed in the hetero-COSY spectrum (Figure 18 , page 139). The assignments of the quaternary carbons, C-1 (δ 140.5 ppm), C-9 (δ 144.5 ppm), C-10 (δ 144.9 ppm) C-7a (δ 129.8 ppm) C-11a (δ 124.2 ppm) and C-1b (δ 127.3 ppm) are achieved by investigating the long range couplings between proton and carbon signals in the COLOC spectrum (Figure 19, page 140). The remaining carbon signals, those of C-1a (δ 119.7 ppm) and C-3a (δ 124.0 ppm) were assigned from the correlations with proton signals visible in its HMBC spectrum (Figure 22 , page 144). Those are as follows:

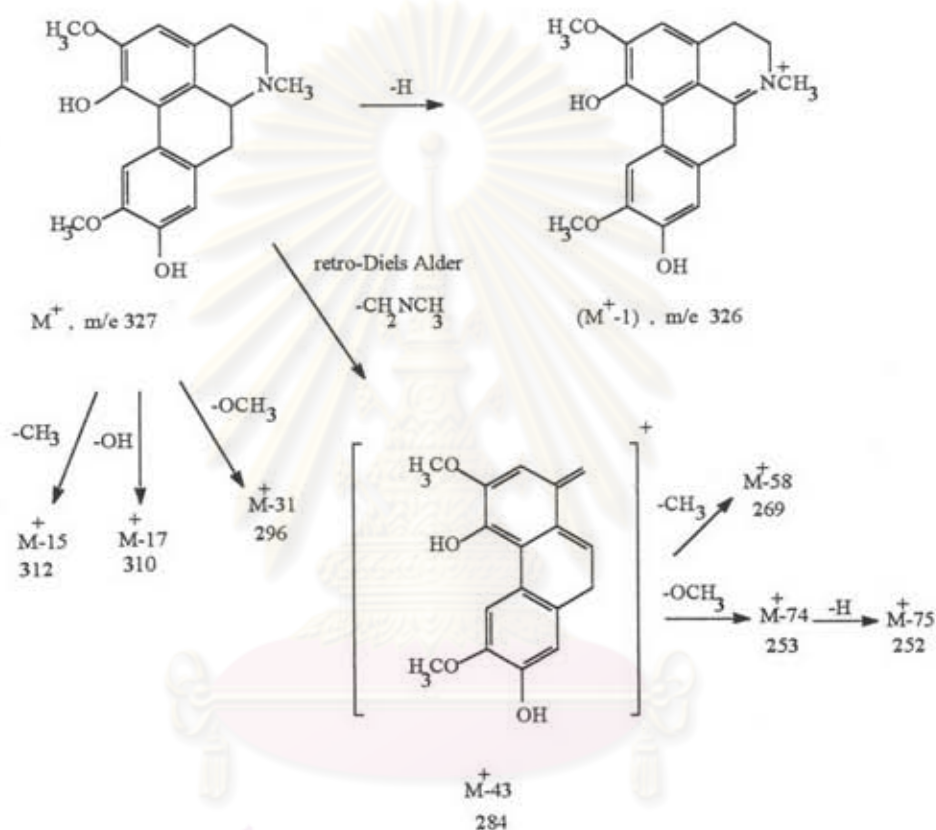
C-1a (δ 119.7 ppm) - H-11(δ 8.01 ppm)

C-3a (δ 124.0 ppm) - H-4 (δ 2.64, 3.14 ppm), H-6(δ 3.04 ppm)

The chemical structure and proton assignments of compound G-47 is shown as follow:



The structure of compound G-47 was also supported by the fragmentation pattern in its eims spectrum. The major fragmentation pathway of this compound are shown as follows:



The complete assignments for all proton and carbon signals are summarized in Table 7.

The unambiguous protons assignment of isoboldine is reported here for the first time. The present identification was in agreement with those assigned chemical shift of isoboldine previously isolated by Gunatilaka and co-workers (1981) and by Guinadeau

and co-worker (1975). The structural and conformations of isoboldine is obtained by conversion into the study on structure of the alkaloid (+)-glaucine (Kerr,1986; Glaser, 1991).

Table 7 ^{13}C and ^1H signal assignments of compound G-47 and long-range correlation between carbons and protons

Position	H(ppm)	C(ppm)	long-range correlation from C on H observed in COLOC spectrum
1	-	140.5	-
1a	-	119.7	-
1b	-	127.3	-
2	-	145.7	-
3	6.54(s)	108.7	C-1
3a	-	124.0	-
4 α	3.14 (ddd, $J=6.1, 12.7, 15.9$ Hz)	28.9	-
4 β	2.63 (dd, $J=3.4, 15.9$ Hz)	-	C-3
5 α	3.02 (d, $J=3.4$ Hz)	53.5	-
5 β	2.51 (dt, $J=3.4, 12.7$ Hz)	-	NCH_3
6a	3.04 (dd, $J=0.9, 4.3$ Hz)	62.5	C-7a

Table 7 cont.

Position	H(δ ppm)	C (δ ppm)	long-range correlation from C on H observed in COLOC spectrum
7 α	2.98 (dd, $J=4.3, 13.7$ Hz)	34.2	C-8
7 β	2.55 (dt, $J=0.9, 13.7$ Hz)	-	C-6a
7a	-	129.8	-
8	6.82(s)	113.9	C-10, C-11a
9	-	144.5	-
10	-	144.9	-
11	8.01(s)	111.6	C-9, C-1a
11a	-	124.2	-
N-CH ₃	2.53(s)	44.0	C-5, C-6a
2-OCH ₃	3.93(s)	56.1	C-2
10-OCH ₃	3.91(s)	56.0	C-10

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