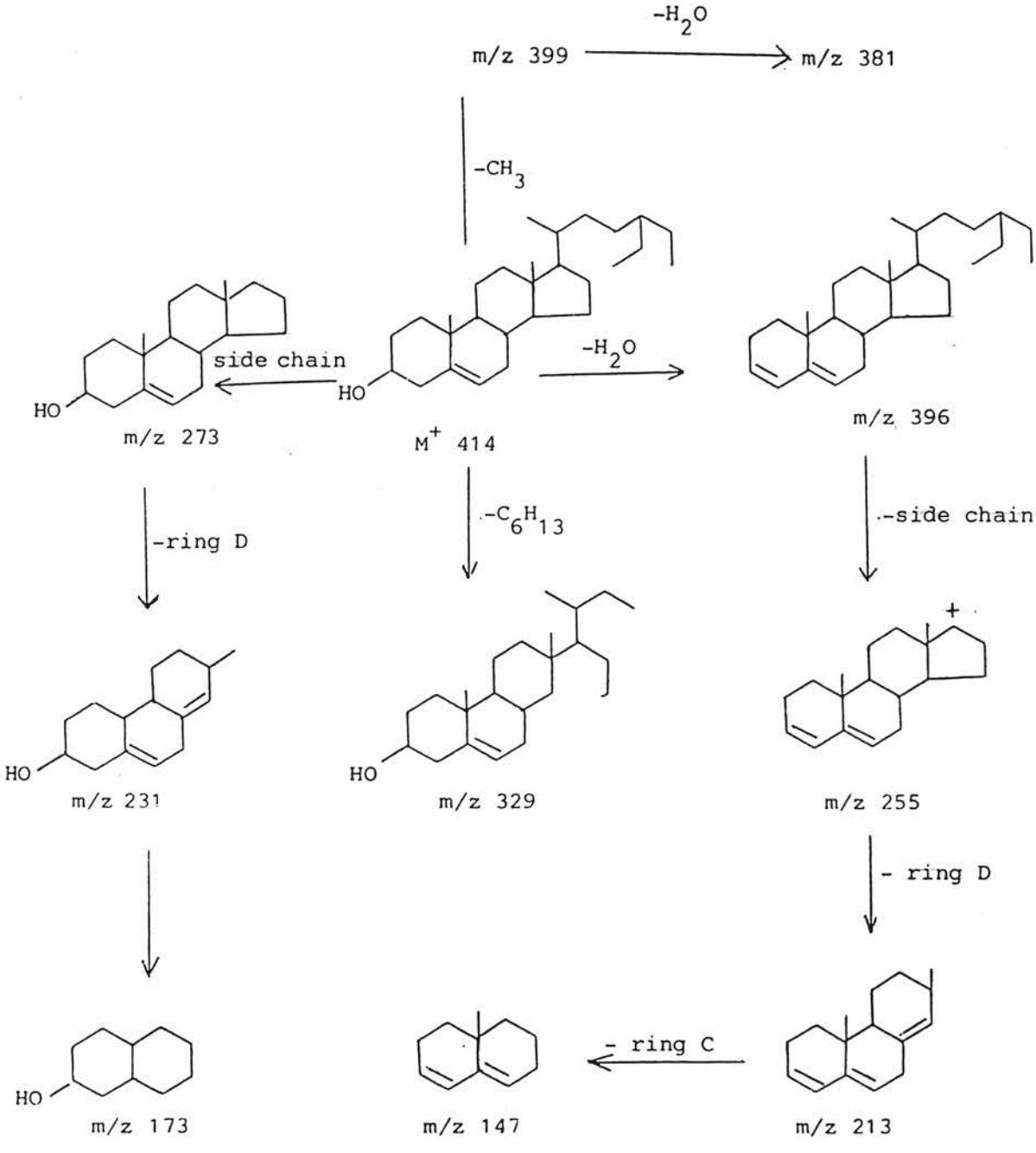
CHAPTER IV

DISCUSSION

The residue from the chloroform extract of the fresh bark of *Michelia longifolia* was separated into four pure components by column chromatography as described in the experimental chapter. The first component eluted was a colorless solid which showed a molecular ion at *m/z* 248 corresponding to the molecular formula $C_{15}H_{20}O_3$. Infrared absorption bands at 1770 and 1650 cm^{-1} suggested the presence of an α-methylene-γ-lactone moiety and the lack of absorption above 3100 cm^{-1} indicated the third oxygen atom was not present as a hydroxyl group. The 400 MHz ^{1}H-nmr spectrum of this component was in good agreement with that previously reported at 60 MHz for the germacranolide epoxide, parthenolide, ML-1 (6). With the aid of a ^{1}H-^{1}H 2D-COSY spectrum ($CDCl_3$), assignment of all the protons in ML-1 was possible. The structure and conformation of ML-1 were previously established unambiguously by single crystal X-ray analysis (98). Determination of the optical rotation of this component from *M. longifolia* established that it was (-)-parthenolide of (6S) absolute configuration as depicted on page 100 (99).

The second component, ML-2 was obtained as a white powder, its IR spectrum revealed the presence of hydroxy and olefinic functions. The electron impact mass spectrum (EIMS) exhibited a weak parent ion at 414 *m/z* corresponding to molecular formula $C_{29}H_{50}O$ and an intense M-H_2O (*m/z* 396) peak which is characteristic of Δ^5-3-β-sterols (100). The proposed fragmentation pattern (101, 102) was shown on page 111.


-H2O
m/z 399
m/z 381
-CH3
side chain
HO
m/z 273
M+ 414
-H2O
m/z 396
-ring D
-C6H13
-side chain
HO
m/z 231
HO
m/z 329
+
m/z 255
- ring D
HO
m/z 173
m/z 147
- ring C
m/z 213

The ^{1}H-NMR spectra was identical to those obtained form a sample of β-sitosterol, which had been isolated from *Typha elephantina*, (97) and also were in accord with other spectra published previously for this sterol (95, 103). Thus, the second component is β-sitosterol.

The third (ML-3) and most polar component was a high-melting, yellow, crytalline solid. Its EIMS spectrum exhibited a strong molecular ion at *m/z* 275 ($C_{17}H_9NO_3$) and its fragmentation pattern was similar to that reported for the alkaloid liriodenine (108). The ^{1}H-nmr (DMSO-d_6 (6) or TFA (104) of ML-3 have been reported previously and are in agreement with these spectra. Liriodenine has limited solubility in chloroform but in experimental section the author reports the 400 MHz ^{1}H-nmr spectra of ML-3 very dilute in $CDCl_3$ and in 10 % DMSO-d_6/$CDCl_3$ solution as previous nmr reports only assigned some of the protons. To assign all the aromatic protons a 2D-COSY experiment (10 % DMSO/$CDCl_3$) was performed. It showed clearly that the doublet for H(11) at δ 8.72 was coupled to the triplet for H(10) at δ 7.77 and that this latter proton was also coupled with H(9) at δ 7.58 ppm. The remaining downfield doublet for H(8) at δ 8.57 ppm was also couple with H(9). This spectroscopic data unambiguously established that the third component was the oxoaporphinoid alkaloid liriodenine, ML-3 has previously been reported to be present in different genera of Magnoliaceae (106).

The fourth component (ML-4) was obtained as white crystalline solid. Its EIMS displayed a parent peak at *m/z* 232 corresponding to the molecular formula $C_{15}H_{20}O_2$. IR absorption bands at 1772 and 1650 cm^{-1} suggested the presence of an α-methylene-γ-lactone moiety.

^{1}H-NMR spectrum showed many similarities to the spectrum of parthenolide, (ML-1), with the only significant differences being in the region of C(4) and C(5). From EIMS, ML-4 has only two oxygen to form γ-lactone that clearly shown from IR absorption band at 1772 cm^{-1}. Thus it is no epoxide group occurring on C(4) and C(5). The two methyl signals at δ1.69 and δ 1.43 are due to protons at C(14) and C(15) which indicated as being interchangeable because of the similar effect of C(1)-C(10) and C(4)-C(5) double bonds. These spectroscopic data unambiguously established that the fourth component was the germacranolide costunolide (6).

As *Michelia longifolia* has been used for medicinal purposes, the biological activities of the constituents isolated from the bark deserve some comment.. Parthenolide, ML-1, displayed significant activity against the human laryngeal epidermoid carcinoma (ED_{50}=0.76) (107) and the 9KB cell culture system (ED_{50}=0.45) (6) while costunolide, ML-4, showed reproducible inhibitory activity against the KB cell culture of a human carcinoma of nasopharynx. Liriodenine, ML-3, showed significant cytotoxicity against the 9KB system (ED_{50}= 1.6 (6) or 3.8 (6)) and also exhibited a wide range of antimicrobial activity *in vitro* (107). The anticarcinogenic activity of β-sitosterol, ML-2, against N-methyl-N-nitrosourea in colon carcinogenesis of animals has been documented also. β-sitosterol is known definitely to inhibit growth of the Walker Carcinosarcoma 256 (subcutaneous) (WA) neoplasm. It is proposed for antineoplastic activity (109). Further work showed it to have some activity against Walker Cancinosarcoma 256 (intramuscular) (WM), Lewis Lung Carcinoma (LL), Murphy-Sturm Lymphosarcoma and marginal activity against Adenocarcinoma 755 (CA) (105).