

CHAPTER IV

DISCUSSION

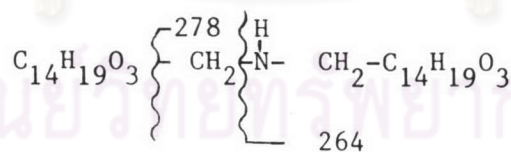
Phytochemical study of the genus *Michelia* (Magnoliaceae) has previously led to the isolation of a variety of alkaloids, sesquiterpene lactones, essential oils and steroids. Most of the sesquiterpene lactones found in *Michelia* spp. are germacranolides having α,β -unsaturated lactone which is *trans*-fused to the C₆-C₇ or C₈-C₇ positions of the carbocyclic skeleton. The presence of α -methylene- γ -lactone in sesquiterpenoid skeleton can enhance the cytotoxic activity of such compounds eg. micheliolide, michelenolide, parthenolide, costunolide, santamarine, dehydrolanuginolide and epitulipinolide diepoxide. Review of chemical constituents in *Michelia* spp. is shown in Table 1.

Fractionation by column chromatography from chloroform extract of the fresh bark of *M. rajaniana* Craib led to obtain alkaloid and several sesquiterpene lactones, four of which are found to contain unusual substituents of germacranolide skeleton.

The white needle crystal (MR-1), was isolated as described in the Experimental section and was found to be the germacranolide epoxide on the basis of the data reported below. MR-1 exhibited IR absorption at 1770 and 1650 cm⁻¹ indicating the presence of α -methylene- γ -lactone functionality. Its mass spectrum showed a weak parent ion at *m/z* 248 which was corresponding to the molecular formula C₁₅H₂₀O₃. The 400 MHz ¹H-NMR spectrum of this component as shown in Table 3 was

in excellent agreement with that of previously reported for the germacranolide epoxide, parthenolide. With the aid of a ^1H - ^1H 2D-COSY spectrum (CDCl_3), assignment of all the protons in MR-1 was possible (Table 3). The structure and conformation of MR-1 were previously established unambiguously by single crystal X-ray analysis (82). Determination of the optical rotation of this component established that it was (-)-parthenolide of (6S) absolute configuration as depicted on page 81.

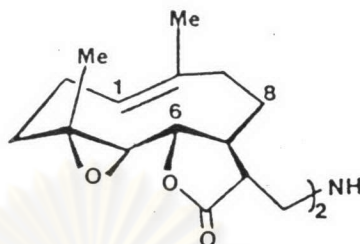
The second component (MR-3), was an amorphous solid and its EIMS (parent peak m/z 513, base peak 278) was consistent with a compound containing two sesquiterpenoid units and one nitrogen atom. Accurate mass determinations of the parent peak and two fragments (m/z 278 and 264, see Experimental) further supported the presence of a nitrogen atom and fragmentations as indicated below. The IR spectrum displayed a strong absorption at 1770 (γ -lactone) and a weak band at 3365 cm^{-1} (N-H). Both the ^1H - and the ^{13}C -NMR spectra (Tables 3 and 4),



respectively showed many similarities to the spectra of parthenolide, (6, 19, 79) with the only significant differences being in the region of C(11) and C(13). In particular, C(11) in MR-3 was a methine carbon (C-H) as determined by the ^{13}C attached proton test (APT) and the two protons on C(13) appeared as an AB pattern ($J = 13.1\text{ Hz}$) at about 3 ppm with additional splitting ($J = 2.8\text{ Hz}$). The chemical shifts of C(13) (46.2 ppm) and of the attached protons were consistent with the presence of a nitrogen atom on that carbon. Assignments for the

protons (2D-COSY experiment) of MR-3 were given in Table 3. On the basis of this spectroscopic information, it was proposed that the second component isolated from *M. rajaniana* is the sesquiterpenoid alkaloid, formed by Michael addition of ammonia to two molecules of parthenolide. The aminomethyl group at C(11) is tentatively assigned the α -configuration because in the $^1\text{H-NMR}$ spectrum of MR-3 in deuterated benzene solvent, H(7) appears as a well-resolved quartet ($J = 8.8$ Hz) as a result of *trans* coupling with H(6), H(8 β) and H(11). Presumably MR-3 is derived from (-)-parthenolide, which has a (6S)-configuration (83), so the same absolute configuration is assigned to this new alkaloid, which the authors have chosen to call bisparthenolidine.

As MR-3 is a new germacranolide derivative, it was interesting in determining its conformation in solution using $^1\text{H-NOE}$ (Nuclear Overhauser Effect) experiments. Low-intensity irradiation of a degassed CDCl_3 solution of MR-3 at 1.67 ppm (the resonance for the C(14) methyl group) caused an increase in the intensity of the signals for H(2 β), H(8 β) and H(9 β) of 43, 43 and 10 %, respectively. In addition, irradiation of the H(6) resonance at δ 3.86 ppm resulted in a 47 and 9 % enhancement of the H(8 β) and C(15) methyl signals, respectively. Since there was interaction between the C(15) methyl group and H(6) but none between H(5) and H(6), the *trans* configuration of the C(4)-C(5) epoxide was confirmed. A 2D-NOE experiment (NOESY) confirmed the interactions mentioned above but also revealed a weak cross-peak correlation between C(14) and C(15) methyl signals, thus indicating the *syn* relationship between these two groups. The NOE results clearly indicate a conformation for this parthenolide derivative as shown below. A similar conformation has been reported for parthenolide (82).



As MR-3 is an unusual natural product, it deserves some comment. From literature citation, MR-3 is the first reported example of a naturally-occurring germacranolide alkaloid, although a piperidine adduct of a pseudoguaianolide (84) and a tertiary amine derived from ammonia and three molecules of α -methylenebutyrolactone (85) have been isolated previously from natural sources. A secondary amine related to MR-3 has been synthesized from ammonia and two molecules of the eudesmanolide, alantolactone, and there have been numerous reports of reactions of secondary amines with the α -methylene group of germacranolides (9, 45, 86)

The third component (MR-4) was isolated as a white crystalline solid. The IR spectrum exhibited absorptions at 3620 (hydroxyl), 1777 (γ -lactone) and 1734 cm^{-1} (ester). The parent peak at m/z 324 was appropriate for the molecular formula $\text{C}_{17}\text{H}_{24}\text{O}_6$, and the fragment at m/z 264, $(\text{M}-\text{HOAc})^+$, suggested the presence of an acetoxy group. The ^1H -NMR spectrum (Experimental) and ^{13}C -NMR spectrum (Table 4), along with the information above, suggested that MR-4 was a dihydroparthenolide containing hydroxyl and acetoxy substituents. In the ^1H -NMR spectrum, H-1 appears as a sharp doublet because of coupling with H-2 β ($J = 10.2$ Hz). Of the two resonances at 4.92 and 4.70 ppm, the more deshielded proton should be attached to the carbon bearing the acetoxy group, and, consequently, the authors would like to propose that the hydroxyl group is at the 2 α position and the H-2 β resonance is at δ 4.70 ppm. Placement of the acetoxy group at C-8 is consistent with the ^1H -NMR spectrum, but, more conclusively, this location results in the anticipated shifts in the ^{13}C -NMR spectrum relative to that of dihydroparthenolide (87). The C-8 resonance is downfield by 42.4 ppm (α effect), the C-7 and C-9 resonances are downfield by 4.8 ppm (β effect), and the C-6 resonance is shifted upfield by 3.5 ppm (γ effect). The 8-acetoxy group is placed in the alpha position by analogy to the ^1H -NMR spectrum of the closely related 8 α -acetoxydihydroparthenolide (26). In the latter compound the H-8 β resonance appears at 4.90, while in MR-4 it is found at 4.92 ppm. In 8-acetoxygermacranolides in which H-8 is alpha, this resonance appears much further downfield at about 5.7 ppm (88). The very

significant deshielding of the C-13 methyl group in MR-4 relative to its position in dihydroparthenolide (δ 18.3 VS δ 13.2, respectively) suggests a *syn*-orientation of this methyl and the acetoxyl group (i.e., both groups alpha). Thus, it is proposed component MR-4 is 8 α -acetoxo-2 α -hydroxydihydroparthenolide, for which the author suggested the name paramicholide. For a related discussion of these assignments, see Jakupovic *et al* (89).

In previous reports on the ^{13}C -NMR spectra of germacranolides, the resonances assigned to C-2, C-3, C-8 and C-9 are usually indicated as being interchangeable (90). Comparison of the spectrum of dihydroparthenolide with those of the oxygenated derivative MR-4 enabled to assign each of these resonances for this family of compounds. Clearly, the resonances at δ 24.0 and δ 36.6 ppm in dihydroparthenolide must be assigned to C-2 and C-3 because introduction of a hydroxyl group at C-9 has little effect on the chemical shifts of these two carbons. The resonance at 36.6 ppm is to C-3 because of the deshielding effect of the neighboring epoxide function. The resonance at 41.1 ppm in dihydroparthenolide is attributed to C-9 because a methylene carbon adjacent to a methyl group on a methyl group on a trisubstituted *trans* double bond, *trans*-(-CH = C(CH₃) CH₂-), has been shown to be significantly deshielded relative to the other allylic carbon. Also, the chemical shifts predicted for these four carbons in MR-4 based on the α , β and γ effects of oxygen substituents on the model system dihydroparthenolide are in excellent agreement with the actual values.

The fourth compound (MR-6) was isolated as white needle crystal. Its EIMS spectrum exhibited a molecular ion at m/e 307 corresponding

to the molecular formula $C_{17}H_{25}NO_4$. The IR spectrum exhibited absorption bands at 3400, 1769 and 1670 cm^{-1} , suggested the presence of N-H, γ -lactone and amide respectively. The $^1\text{H-NMR}$ (400 MHz) spectrum of MR-6 indicated similar pattern to that of parthenolide. A broad signal at δ 6.31 and singlet at δ 2.02 were assigned to N-H and $\overset{\text{O}}{\parallel}\text{C-CH}_3$ respectively. This was confirmed by $^{13}\text{C-NMR}$ that two carbon signals indicated the N-acetyl of parthenolide nucleus. Confirmation of the chemical shift of the C'_1 and C'_2 resonances by using APT technique showed that an oxygenated sp^2 carbon at δ 170.7 ppm indicated C'_1 carbonyl and δ 23.9 ppm indicated C'_2 methyl. Differentiations of C_{11} and C_{13} with those of parthenolide were remarkable. It was shown for C_{11} and C_{12} resonances in MR-6 as sp^3 carbon while in parthenolide was a sp^2 carbon. Thus, it is concluded that MR-6 is N-acetylparthenolidine, a new germacranolide.

The fifth component (MR-7) was isolated as colourless crystalline solid. The IR spectrum suggested the presence of a γ -lactone (1968), a hydroxyl group (3420-3240), a N-H group (3500) and an amide (1658 cm^{-1}). The molecular weight was 323 corresponding to the molecular formula $C_{17}H_{25}NO_5$. The $^1\text{H-NMR}$ (400 MHz) spectral data was similar to those obtained from MR-6 except for a few points as indicated in the following. Hydroxyl signal appeared as doublet while NH appeared as a doublet of doublet (coupling with H_8 and H_{13a} , H_{13b} respectively). Study on $^{13}\text{C-NMR}$ spectrum showed significant chemical shift of C-8 to lower field at 72.3 ppm indicating a hydroxyl group attached to this carbon. Thus, it is concluded that MR-7 is N-acetyl-8 α -hydroxyparthenolidine, also a new germacranolide.

The sixth component (MR-8) and most polar component was a high-melting, yellow, crystalline solid. Its EIMS exhibited a strong

molecular ion at m/z 217 ($C_{17}H_9NO_3$) and its fragmentation pattern was similar to that reported for the alkaloid liriodenine. Also, the IR and UV spectra (91) for this component were the same as those previously reported for liriodenine. The 1H -NMR (DMSO- d_6 or TFA (92)) and ^{13}C -NMR spectra (93) of MR-8 have been reported previously and are in agreement with these spectra. Liriodenine has limited solubility in chloroform but in experimental the author reported the 400 MHz 1H -NMR spectra of MR-8 in very dilute solution of $CDCl_3$ 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 8.72 was coupled to the triplet for H(10) at 7.77 and that this latter proton was also coupled with H(9) at 7.58 ppm. The remaining downfield doublet for H(8) at 8.57 ppm was also coupled with H(9). This spectroscopic data unambiguously established that the sixth component was the oxoaporphinoid alkaloid liriodenine. Liriodenine has previously been reported to be present in a number of different genera of Magnoliaceae(see table 1 p.9).

Sesquiterpene lactones are common constituents of most genera of the Compositae, with the exception of the evolutionary "advanced" tribe, the Tagetae. Eudesmanolides, similar to those found in the genus *Inula* (Compositae), are also present in the liverworts (Hepaticae), *Frullania tamarisci* (80, 81). This present investigation has led to isolation and characterization of eudesmanolides occurring in *Grangea maderaspatana* (Compositae), a well known indigenous drug of Thailand. Three eudesmanolides were found to form *cis*-lactone ring junction.

The first component (GM-1) was isolated as a crystalline solid which exhibited IR spectrum at 1767cm^{-1} (γ -lactone). Its EIMS showed a molecular ion at m/z 232 corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_2$. Evidences from the ^1H -NMR spectrum (400 MHz) have shown that GM-1 was *cis*-lactone. The major differences from *trans*-lactone were in the coupling of the exomethylene protons (δ 6.14, 5.57, both d, J 1 Hz in *cis*-lactone, δ 6.13, 5.43, both d, J 3 Hz (81) in *trans*-lactone) and in the signal for H-6 which appeared as a doublet at δ 5.26 J 8.9 Hz for GM-1 while a diffuse doublet at higher field (δ 4.54 for *trans*-lactone (81)). Irradiation at δ 5.26 (H-6) changed the signal at δ 2.95 (H-7) to a broadened triplet consistent with the presence of an adjacent methylene group. The reverse experiment caused H-6 to collapse to a broad singlet. The residual broadening of H-6 is due to homoallylic coupling with the vinyl methyl group and this was demonstrated by irradiating H-6 whereupon the intensity of the vinyl methyl signal increased. From the magnitude of this residue coupling the stereochemistry of the lactone ring junction was deduced to be *cis* (81). All these data are consistent with GM-1 being frullanolide (81).

The second component (GM-2) was isolated as crystalline solid. The EIMS showed a molecular ion at m/z 248 corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_3$. Infrared absorption bands at 1771 and 3580cm^{-1} suggested the presence of α -methylene- γ -lactone moiety and hydroxyl group. Both ^1H and ^{13}C -NMR spectra showed many similarities to those of frullanolide, with the only significant differences being in the region of C(7). In particular, C(7) in GM-1 was a methine carbon (C-H) (-41.2 ppm) as determined by the ^{13}C attached proton test (APT). The chemical shift of C(7) to lower field (76.0) in GM-2 was consistent with the

presence of a hydroxyl group on that carbon. $^1\text{H-NMR}$ signal for the C-6 methine proton appeared as singlet which suggested the presence of substituent on C-7. Thus, it is concluded that structure of **GM-2** is a new and unusual 7-hydroxy eudesmanolide called 7-hydroxyfrullanolide.

The third component (GM-3) obtained as crystalline solid, its EIMS exhibited molecular ion at m/z 266 corresponding to molecular formula $\text{C}_{15}\text{H}_{22}\text{O}_4$. The IR spectrum exhibited absorption bands at 3750 and 1770 cm^{-1} suggested the presence of hydroxyl and γ -lactone respectively. $^1\text{H-NMR}$ spectrum was similar to that obtained from GM-2, with the only few points should be discussed. H-13 (3H) appeared to be doublet while H-11 appeared as quartet. This evident was confirmed that at C-11 and C-13 region was dihydrofrullanolide. Methine proton at C-3 appeared as doublet of doublet at δ 4.0 because of the two protons coupling ($\text{H}_{2\alpha}$ and $\text{H}_{2\beta}$) while carbon resonance at -69.6 ppm suggested the introduction of hydroxyl group on this carbon. Thus, it is concluded that GM-3 was 3α -, 7α -dihydroxy dihydrofrullanolide, a new and unusual eudesmanolide.

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