

CHAPTER IV

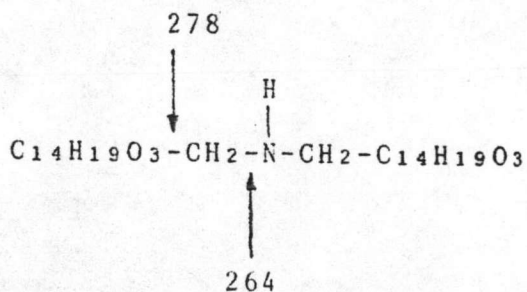
DISCUSSION

Chromatographic purification of the alcoholic extract of *Paramichelia baillonii* as described in the Experimental, provided the four components to be discussed. The electron impact mass spectrum (eims) of PB-1 showed a molecular ion at m/z 250 corresponding to the molecular formula $C_{15}H_{22}O_3$. An infrared absorption at 1775 cm^{-1} suggested the presence of a γ -lactone and the ^1H -nmr spectrum and optical rotation established clearly that PB-1 was (-)-dihydroparthenolide. The 400 MHz ^1H -nmr spectrum of PB-1 is reported in Table III as previously only low resolution spectra with few proton assignments were reported (2,148). The ^{13}C spectrum of PB-1 is reported for the first time in Table IV. (-)-Dihydroparthenolide has been isolated previously from *Michelia lanuginosa* (308), *M. compressa* (2) and *Ambrosia artemisiifolia* L. (309).

The electron impact mass spectrum (eims) of component 2 (PB-2) showed a molecular ion at m/z 248 corresponding to the molecular formula $C_{15}H_{20}O_3$. Infrared bands at 1770 and 1650 cm^{-1} suggested the presence of an α -methylene- γ -lactone moiety and the 400 MHz ^1H -nmr spectrum of

this component was in good agreement with that previously reported at 60 MHz (2,148) and at 200 MHz for the germacranolide epoxide, parthenolide. The 200 MHz assignments for PB-2 are included in Table III for comparison with PB-1 and PB-3 and in a footnote it is reported additional assignments obtained from 400 MHz spectrum and an ^1H - ^1H 2D-COSY experiment. The previously reported ^{13}C spectrum of PB-2 (310) is included in Table 4 for comparison with the spectra of PB-1 and PB-3, also. The structure and conformation of PB-2 were previously established unambiguously by single crystal X-ray analysis (311). Determination of the optical rotation of this component from *P. baillonii* established that it was (-)-parthenolide of (6S) absolute configuration as depicted in page 85 (312).

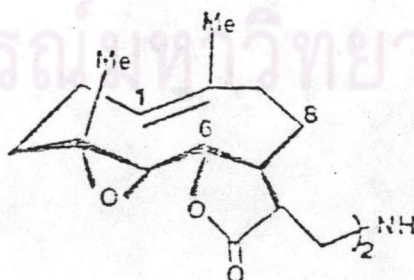
The third component, PB-3 was a crystalline solid and its eims (parent peak m/z 513, base peak 278) was consistent with a compound containing two sesquiterpenoid units and one nitrogen atom. Accurate mass determinations of the parent peak and two fragments (m/z 278 and 264, see Experimental) further supported the presence of a nitrogen atom and fragmentation 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 (Table III and IV respectively) showed many similarities to the spectra of dihydroparthenolide (PB-1) with the only significant differences being in the region of C(13). In particular, the two protons on C(13) in PB-3 appeared as an AB pattern ($J = 13.1 \text{ Hz}$) at about 3 ppm with additional splitting ($J = 2.8 \text{ Hz}$) and in the ^{13}C spectrum the chemical shift of C(13) (46.2 ppm) and the attached proton test (APT) were consistent with the presence of a nitrogen atom on a methylene carbon. Assignments for the protons of PB-3 given in Table III were aided by a 2D-COSY experiment. On the basis of this spectroscopic information it is proposed that this component is the sesquiterpenoid alkaloid PB-3, 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 ^1H -nmr spectrum of PB-3 in benzene solvent, H(7) appears as a well-resolved quartet ($J = 8.8 \text{ Hz}$) as a result of *trans* couplings with H(6), H(8 β) and H(11). Presumably PB-3 is derived from (-)-parthenolide, which has a (6S)-configuration (313), so the same absolute configuration is assigned to this new alkaloid, which have been chosen to call bisparthenolidine.

As PB-3 is a new germacranolide derivative, it is of interest in determining its conformation in solution using ^1H -NOE (nuclear Overhauser effect) experiments. Low-inten-

sity irradiation of a degassed CDCl_3 solution of PB-3 at 1.67 ppm (the resonance for the C(14) methyl group) cause an increase in the intensity of the signals for H(2β), H(8β) and H(9β) of 43, 43 and 10%, respectively. In addition, irradiation of the H(6) resonance at 3.86 ppm resulted in a 47 and 9% enhancement of the H(8β) 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 the 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 in PB-3A. A similar conformation has been reported for parthenolide (PB-2) (311).



PB-3A

As PB-3 is a new and unusual natural product it deserves some additional comment. The ^{13}C -nmr spectrum of PB-3 shows only fifteen lines as would be expected for one pure diastereomer; a mixture of diastereomers would be expected if the component was formed chemically rather than in the plant. To our knowledge, PB-3 is the first reported example of a naturally-occurring germacranolide alkaloid, although a piperidine adduct of a pseudoguianolide (313) and a tertiary amine derived from ammonia and three molecules of α -methylenebutyrolactone (314) have been isolated previously from natural sources. A secondary amine related to PB-3 has been synthesized from ammonia and two molecules of the eudesmanolide, alantolactone (315), and there have been numerous reports of reactions of secondary amines with the α -methylene group of germacranolides (316).

The fourth and most polar component, PB-4, isolated from *P. baillonii* was a high-melting, yellow, crystalline solid. Its ei mass spectrum exhibited a strong molecular ion at m/z 217 ($\text{C}_{17}\text{H}_9\text{NO}_3$) and its fragmentation pattern was similar to that reported for the alkaloid liriodenine (317). Also, the ir (318,319) and UV spectra (319,320) for this component were the same as those previously reported for liriodenine. The ^1H -nmr [DMSO- d_6 (2) or TFA (321)] and ^{13}C -nmr spectra (322) of PB-4 have been reported previously and are in agreement these investigated spectra. Liriodenine has some solubility in 10% DMSO- d_2 /CDCl $_3$ and in the Experi-

mental we report the 400 MHz ^1H -nmr spectra of PB-4 in this solvent as previous nmr reports only assigned some of the protons. To assign all the aromatic proton a 2D-COSY experiment 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 fourth component was the oxoaporphinoid alkaloid liriodenine, PB-4. PB-4 has previously been reported to be present in a number of different Magnoliaceae genera (323,324).

Since *P. baillonii* has been used by Thai natives for medicinal purposes, some of the biological activities of the components isolated are noted below. Parthenolide, PB-2, demonstrates significant activity against the human laryngeal epidermoid carcinoma ($\text{ED}_{50}=0.76$ mcg/ml) (176) and the 9KB cell culture system ($\text{ED}_{50}=0.45$ mcg/ml) (2) while dihydroparthenolide is inactive in the latter assay (2). Liriodenine, PB-4, shows significant cytotoxicity against the 9KB system [$\text{ED}_{50}=1.6$ mcg/ml(325) or 3.8 mcg/ml(2)] and also exhibits a wide range of antimicrobial activity *in vitro* (326). In the present study, the cytotoxicity of the new alkaloid bisparthenolidine, PB-3, was examined and was found to have a significant $\text{ED}_{50}=0.73$ mcg/ml in the KB cell

culture assay. Normally Micheal addition such as in PB-3, would be expected to reduce or destroy the biological activity of the compound (248), but the plant may be using this adduct as a storage mechanism and upon β -elimination of the amino group, the active PB-2 is generated (248).



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