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CONSTITUENTS OF *MICHELIA RAJANIANA*. TWO NEW GERMACRANOLIDE AMIDES[1]

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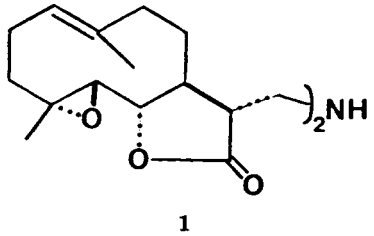
ABSTRACT.—Six components have been isolated from the bark of *Michelia rajaniana*, and their structures have been determined by spectroscopic analysis. Four of the components have been reported previously: The germacranolide (−)-parthenolide and the oxoaporphinoid alkaloid liriodenine have been observed as constituents of several species, and (−)-bisparthenolidine [**1**] and (+)-paramicholide [**2**] have been reported recently by us as constituents of *Paramichelia baillonii*. The two new components **3** and **4**, which are novel derivatives of parthenolide and contain an unusual *N*-acetyl substituent at C-13, have been given the names (+)-*N*-acetylparthenolidine and (+)-*N*-acetyl-8α-hydroxyparthenolidine, respectively. The crude $CHCl_3$ extract of the bark of *M. rajaniana* exhibited strong cytotoxicity in the KB cell culture assay.

The species *Michelia rajaniana* Craib of the Magnoliaceae family is endemic to northern Thailand (2) and is known to the natives as "Champi luang" (2,3). The timber of *M. rajaniana* is used extensively in the plywood and furniture industries (4), and the bark has been used medicinally as a substitute for *Michelia champaca* L. A decoction prepared from the bark of the latter species has been used as a febrifuge, as a protective medicine for mothers after childbirth (5,6), and in India for the treatment of abdominal tumors (7). There have been no previous phytochemical studies on any part of *M. rajaniana*, and in this report we describe the structural elucidation of six components isolated from the bark of the plant.



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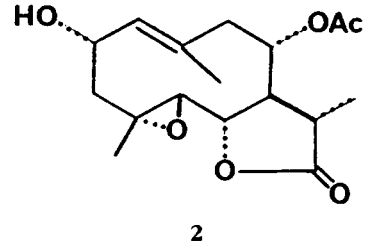

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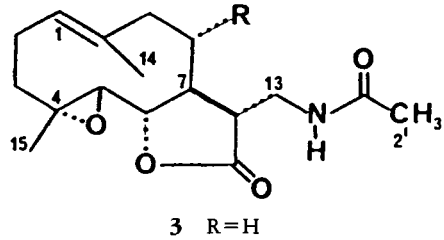

3 R=H
4 R=OH

[1]Part IX in the series of "Studies on Thai Medicinal Plants." For Part VIII, see Likhitwitayawuid *et al.* (1).

Chromatographic purification of the alcoholic extract of the bark as outlined in the Experimental section gave six components, which will be discussed in the order they were eluted from the column. The least polar component was found to be identical to (−)-parthenolide, an epoxy germacranolide we identified previously as a constituent of *Paramichelia baillonii* (8). The second component was shown to be identical to bisparthenolidine [**1**], a compound first isolated by us from *P. baillonii* (8). This unusual germacranolide alkaloid was found to have significant cytotoxicity in the KB cell culture assay (8). The previously reported ^{1}H- and ^{13}C-nmr spectra of **1** are provided (Tables 1 and 2, respectively) for comparison with the new compounds **3** and **4**. The third component was found to be (−)-paramicholide [**2**], a substituted dihydroparthenolide which was isolated first from *P. baillonii* (9). The optical rotation of **2**, which was not reported in our previous study, is given in the Experimental section.

The ir spectrum of the fourth component, **3**, exhibited absorptions at 1770 (s) (γ-lactone), 1670 (s), and 3442 cm^{-1} (w) (secondary amide). The accurate mass of the parent peak at *m/z* 307 in the eims of **3** was consistent with the molecular formula $C_{17}H_{25}NO_4$, and the *m/z* 248 fragment $[M - H_2NAc]^+$ suggested the presence of an NH-acetyl moiety which was expelled via a McLafferty-type rearrangement. A comparison of the ^{1}H- and ^{13}C-nmr spectra of **1** and **3** (Tables 1 and 2, respectively) suggested that these compounds were closely related, with the major differences being in the region of C-13 and the presence of acetyl resonances in **3**. Thus, we believe this component possesses the structure depicted in **3** and suggest the name (+)-*N*-acetylparthenolidine for this new germacranolide alkaloid. (The names for **1**, **3**, and **4** are based on the hypothetical compound parthenolidine, which is 13-aminodihydroparthenolide.) The nmr spectral data for **3** indicate that the *N*-acetyl group adopts the conformation shown in Figure 1 with the amide proton hydrogen bonding to the carbonyl

TABLE 1. ^{1}H-nmr Spectra of **1**, **3**, and **4**.[a]

Proton	Compound		
	1	**3**	**4**
1	5.27 (dd, 9.8, 2.2)	5.17 (br d, 9.7)	5.17 (br d, 9.1)
2α	2.26 (dd, 12.1, 6.0)	2.17 (m)	2.10 (m)
2β	2.40 (m)	2.40 (m)	2.35 (m)
3α	1.23 (td, 13.9, 5.9)	1.21 (ddd, 13.0, 13.0, 6.0)	1.15 (ddd, 12.9, 12.9, 6.6)
3β	1.88 (dd, 14.6, 5.9)	2.10 (m)	2.45 (ddd, 12.9, 12.9, 7.8)
5	2.74 (d, 8.8)	2.70 (d, 8.9)	2.69 (d, 8.7)
6	3.86 (dd, 8.8, 8.8)	3.87 (dd, 8.9, 8.9)	3.94 (dd, 8.7, 8.7)
7	2.40 (m)	2.40 (m)	2.38 (m)
8α	2.18 (m)	2.20 (m)	—
8β	1.70 (m)	1.25 (m)	3.76 (ddd, 11.2, 9.3, 9.3)
9α	2.10–2.18 (m)	2.20 (m)	2.35 (m)
9β			2.65 (br d, 12.7)
11	2.40 (m)	2.40 (m)	2.99 (dd, 11.2, 5.2)
13α	3.15 (dd, 13.1, 2.8)	3.66 (ddd, 14.1, 6.5, 3.5)	4.10 (ddd, 12.9, 6.5, 5.2)
13β	2.92 (dd, 13.1, 2.8)	3.53 (ddd, 14.1, 6.5, 6.3)	3.58 (dd, 12.9, 6.5)
14	1.67 (s)	1.69 (s)	1.72 (s)
15	1.30 (s)	1.30 (s)	1.28 (s)
N-H	—	6.31 (br dd, 6.5, 6.5)	6.81 (dd, 6.5, 6.5)
Ac	—	2.02 (s)	2.08 (s)
OH	—	—	4.95 (d, 11.2)

[a]Chemical shifts are in ppm from TMS, coupling constants are in parentheses in Hertz, and the samples were dissolved in $CDCl_3$.

TABLE 2. ^{13}C-nmr Spectra of **1**, **3**, and **4**.[a]

Carbon	Compound		
	1	**3**	**4**
1	125.3(−)	125.1(−)	127.3(−)
2	24.2(+)	24.1(+)	24.4(+)
3	36.5(+)	36.3(+)[b]	35.8(+)
4	61.6(+)	61.7(+)	61.9(+)
5	66.1(−)	66.2(−)	66.0(−)
6	82.3(−)	82.9(−)	78.9(−)
7	49.0(−)	46.6(−)	51.5(−)
8	30.2(+)	29.8(+)	72.3(−)
9	40.9(+)	40.9(+)	52.4(+)
10	134.3(+)	134.6(+)	130.3(+)
11	45.5(−)	48.6(−)	46.8(−)
12	176.7(+)	176.6(+)	177.0(+)
13	46.2(+)	36.6(+)[b]	39.6(+)
14	17.2(−)[b]	17.2(−)[c]	17.5(−)[b]
15	16.8(−)[b]	16.9(−)[c]	17.3(−)[b]
1′	—	170.7(+)	173.2(+)
2′	—	23.2(−)	22.9(−)

[a]Chemical shifts are in ppm from TMS, solvent was $CDCl_3$, and (+) and (−) are signs from the attached proton test.

[b,c]Assignments in the same column with the same superscripts may be interchanged.

oxygen of the lactone. In the ^{1}H-nmr spectrum of **3** this amide proton appears as a downfield doublet of doublets because of different couplings with H-13α and H-13β, which are diastereotopic. Decoupling experiments were performed to confirm these assignments. In the ^{13}C-nmr spectra, the C-13 resonance in **3** is shielded relative to the corresponding signal in **1** (36.6 vs 46.2 ppm, respectively) as would be expected for a *syn* orientation of the amide carbonyl group and C-13 (Figure 1) (10,11). Interestingly, the H-13 protons in **3** are deshielded relative to those in **1**. A similar reversal of shifts for the carbon and hydrogen resonances (i.e., carbon shielded while the attached protons were deshielded) in amides has been reported previously (12). To our knowledge, component **3** is the first reported example of a naturally occurring germacranolide amide.

The fifth component exhibited ir absorptions at 1768 (γ-lactone) and 1656 (amide) as well as a broad peak at 3300 cm^{-1} (OH and NH). The mass spectrum and the elemental analysis both were consistent with the molecular formula $C_{17}H_{25}NO_5$. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2, respectively), along with the above information, suggested that **4** was a parthenolidine amide derivative containing an hydroxyl sub-

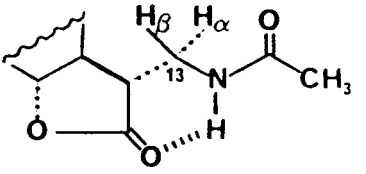

FIGURE 1. Partial structure of **3** showing the conformation of the *N*-acetyl group.

stituent. Most of the proton signals in the 400 MHz ^{1}H-nmr spectrum of **4** were clearly resolved and rich in detail. A ^{1}H-^{1}H 2D COSY experiment and individual decoupling experiments were employed to confirm the assignments reported in Table 1, and a few of these results will now be described. Irradiation of the signal for N-H at 6.81 ppm resulted in collapse of the multiplet for H-13α at 4.10 to a doublet of doublets and collapse of the broadened doublet of doublets for H-13β at 3.58 ppm to a broadened doublet. Irradiation of H-13α caused sharpening of the N-H signal and simplification of the H-13β resonance but, more importantly, resulted in collapse of the doublet of doublets for H-11 at 2.99 ppm to a doublet (J = 11.2 Hz). This large coupling between H-7 and H-11 clearly established the *trans* stereochemistry of these two protons and confirmed the α orientation of C-13 and the attached amide function. Irradiation of the doublet for the hydroxyl proton at 4.95 ppm changed the broadened quartet (actually a ddd) for H-8 at 3.76 to a broadened triplet (actually a dd). Also, when the ^{1}H-nmr spectrum of **4** was obtained in $CDCl_3$ containing a trace of acid, the hydroxyl proton signal was shifted upfield to about 2.3 ppm (disappeared on addition of D_2O) because of proton exchange, and the H-8 resonance was a triplet rather than a quartet. This triplet at 3.76 ppm (J = 9.3 Hz) is appropriate for *trans* couplings of H-8β with H-9α and H-7 and, thus, confirms the placement of the OH group in the α position. In other germacranolides with an 8α-OH, H-8β also appears at about 3.8 (13), while in related compounds with an 8β-OH, the H-8α resonance appears much further downfield at about 4.7 ppm (14). All of these decoupling experiments, plus additional ones not discussed here, support the structure proposed for **4**. Comparison of the ^{13}C-nmr spectra of **3** and **4** (Table 2) was very instructive also. It was reported previously (15) that a hydroxy group produces a pronounced downfield shift (about 40 ppm) of the carbon to which it is attached (α effect), a smaller downfield shift (5–10 ppm) of the β carbon, and an upfield shift (a few ppm) of the γ carbon. The resonance for C-8 in **4** is shifted downfield by 42 ppm (α effect) as compared to the same carbon in **3** because of the hydroxyl group in the former. Likewise, C-7 and C-9 in **4** are shifted downfield by 5 and 11 ppm (β effect), respectively, relative to the same carbons in **3**. On the other hand, C-6, C-10, and C-11 in **4** are all shifted upfield by about 4, 4, and 2 ppm (γ effect), respectively, relative to **3**. Thus, the ^{13}C-nmr results are completely consistent with the structure proposed for **4**, which is another previously unreported germacranolide amide for which we propose the name (+)-*N*-acetyl-8α-hydroxyparthenolidine.

The sixth and most polar component isolated from *M. rajaniana* was a high-melting, yellow, crystalline solid. Comparison of this component with a sample isolated previously from *P. baillonii* (8) established that this compound was the alkaloid liriodenine.

The close chemotaxonomic relationship between *M. rajaniana* and *P. baillonii* (8) should be noted. Both species contain the germacranolide epoxide parthenolide, the unusual germacranolide alkaloid (−)-bisparthenolidine [**1**], the polysubstituted dihydroparthenolide (+)-paramicholide [**2**], and the alkaloid liriodenine. Parthenolide (16,17), bisparthenolidine (8), and liriodenine (16) have all been reported to exhibit significant antitumor activity. The crude $CHCl_3$ extract of *M. rajaniana* (see Experimental) was subjected to the KB cell culture assay and was found to exhibit strong cytotoxicity based on the National Cancer Institute, USA (NCI) standard. The new germacranolide amides **3** and **4** were tested in the human tumor cell lines A-549 (lung), MCF-7 (breast), and HT-29 (colon) and were found to be inactive.

EXPERIMENTAL

INSTRUMENTS.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WH-400 spectrometer with TMS (δ = 0) as internal standard and $CDCl_3$ as solvent. The multiplicities for the ^{13}C spectra were deter-

mined by the attached proton test which produced positive (+) quaternary C and CH_2 signals and negative (−) CH and CH_3 signals. Ir spectra were obtained on a Nicolet Model 20 SX/C Ft-ir spectrometer and mass spectra on a VG Micromass 7070F or a ZAB-E spectrometer. Tlc analyses were performed on Si gel GF-254 plates of thickness 0.25 mm. Optical rotations were performed in a Bendix NPL automatic polarimeter.

PLANT MATERIAL.—The bark of *M. rajaniana* was collected from Doi Suthep, Chiang Mai Province, Thailand in August 1985. Authentication was achieved by comparison with herbarium specimens at the Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperative, Thailand. A voucher specimen of plant material has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

EXTRACTION AND PURIFICATION.—The fresh bark of *M. rajaniana* (3 kg) was macerated twice with 95% EtOH (10 and 15 liters) over a 3-day period, and the suspensions were filtered. The combined filtrates were evaporated under reduced pressure, H_2O (5 liters) was added to the residue, and the mixture was extracted with $CHCl_3$ (8 × 500 ml). The combined extracts were dried (anhydrous Na_2SO_4) and the solvent removed to yield 12.5 g of a syrupy residue. Purification of the residue was effected using Si gel chromatography with C_6H_6-EtOAc (1:1) as eluent and collecting 25-ml fractions. Fractions 1–13, 17–39, and 41–53 afforded, after evaporation, residues A, B, and C, respectively. Residue A was rechromatographed on Si gel using C_6H_6-Me_2CO (6:1) to provide 138 mg of parthenolide and 79 mg of **1**. Similarly, residue B with $CHCl_3$-Me_2CO (6:2) gave 84 mg of **2**, 80 mg of **3**, and 126 mg of a fraction which was found by ^1H nmr to be a mixture of at least three components and is still under investigation. Purification of residue C with $CHCl_3$ solvent yielded 344 mg of **4** and 150 mg of liriodenine.

(−)-PARTHENOLIDE.—Rotation, ir, ^1H and ^{13}C nmr, and eims agree with previously reported values (8).

(−)-BISPARTHENOLIDINE [**1**].—Mp, rotation, ir, and eims agree with previously reported values (8); ^1H and ^{13}C nmr see Tables 1 and 2, respectively.

(−)-8α-ACETOXY-2α-HYDROXYDIHYDROPARTHENOLIDE (PARAMICHOLIDE) [**2**].—Ir, eims, ^1H and ^{13}C nmr agree with previously reported values (9); $[\alpha]^{24}D$ −47° ($c = 0.76$, $CHCl_3$).

(+)-*N*-ACETYLPARTHENOLIDINE [**3**].—Tlc [MeOH-EtOAc-petroleum ether (1:3:6)] R_f 0.37; $[\alpha]^{24}D$ +23° ($c = 0.67$, $CHCl_3$); ir ν max ($CHCl_3$) 3442, 3010, 2928, 1770 (s), 1670, 909 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 307 (25), 289 (11), 279 (10), 264 (11), 248 (14), 131 (100); hrms *m/z* 307.1788 $[M]^+$ (calcd for $C_{17}H_{25}NO_4$, 307.1783).

(+)-*N*-ACETYL-8α-HYDROXYPARTHENOLIDINE [**4**].—Mp 188–190°; tlc [MeOH-EtOAc-petroleum ether (1:3:6)] R_f 0.31; $[\alpha]^{20}D$ +51° ($c = 0.75$, $CHCl_3$); ir ν max ($CHCl_3$) 3300 br, 2932, 1768, 1656, 1073, 973 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M - H_2O]^+$ 307 (4), 256 (100), 214 (39), 156 (64). Calcd for $C_{17}H_{25}NO_5$: C 63.14, H 7.79, N 4.33; found C 62.57, H 7.96, N 4.19.

LIRIODENINE.—Mp, ^1H nmr, and eims agree with previously reported values (8).

CYTOTOXICITY ASSAY.—The KB cell culture assay was performed at the National Cancer Institute in Bangkok. The crude $CHCl_3$ extract from the bark of *M. rajaniana* was found to exhibit strong cytotoxicity based on the NCl standard. Amides **3** and **4** were tested at the Purdue Cancer Center Cell Culture Laboratory, Department of Medicinal Chemistry and Pharmacognosy, Purdue University, using human tumor cell lines A-549 (lung), MCF-7 (breast), and HT-29 (colon), and both compounds were found to be inactive [ED_{50} (μg/ml)>100].

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