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CONSTITUENTS OF *PARAMICHELIA BAILLONII*. THREE NEW GERMACRANOLIDES[1]

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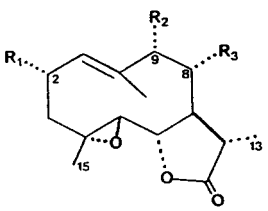
In a previous report (1) we described the isolation and identification of four components obtained from the bark of *Paramichelia baillonii* (Pierre) Hu (Magnoliaceae), a species used by the natives of northern Thailand for medicinal purposes. The components identified were the germacranolide epoxides (−)-dihydroparthenolide and (−)-parthenolide, the oxoaporphinoid alkaloid liriodenine, and a new germacranolide alkaloid which we named (−)-bisparthenolidine. In this paper we describe the structural elucidation of three new germacranolides which also were isolated from the bark of *P. baillonii*.

In our previous study of this species (1) we mentioned a chromatographic fraction that eluted after bisparthenolidine but was not investigated further. ^{1}H-nmr analysis of this fraction showed it was a 2:1:1 mixture of components **1**, **2**, and **3**, respectively. The three components were separated by flash chromatography, and the structural elucidation of each is described herein. Compound **1** exhibited ir absorptions at 3472 (broad) and 1777 cm^{-1}, indicating the presence of hydroxyl and γ-lactone functionalities, respectively. Its mass spectrum showed a weak parent ion at *m/z* 266 (the exact mass was consistent with the molecular formula $C_{15}H_{22}O_4$) and also showed an $[M-18]^+$ fragment, presumably from dehydration of the alcohol. Examination of the ^{1}H-nmr spectrum of **1** revealed many similarities to the spectrum of dihydroparthenolide [**4**] (see Table 1 for comparisons). The ^{1}H- and ^{13}C-nmr spectra were most instructive in determining the position and stereochemistry of the hydroxyl group that had been indicated by the ir and ms spectra. A comparison of the ^{13}C-nmr spectra of **1** and the model compound **4** (Table 2) indicated the major differences were in the region of C-9. It was established previously (2) that a hydroxyl group produced 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. In the ^{13}C spectrum of **1**, the C-9 resonance is shifted downfield by 38.5 ppm relative to **4**, C-8 is shifted downfield by 8.1 ppm, and C-7 (the γ-carbon) is shifted upfield by 3.4 ppm. These shifts establish that the hydroxyl group is on C-9. To determine the configuration of the hydroxyl group, a comparison of the ^{1}H-nmr spectrum of 9α-hydroxy-



1 $R_1=R_3=H$, $R_2=OH$
2 $R_1=OH$, $R_2=H$, $R_3=OAc$
3 $R_1=OH$, $R_2=R_3=H$
4 $R_1=R_2=R_3=H$

[1]Part VI in the series "Studies on Thai Medicinal Plants." For Part IV, see Ruangrungsi *et al.* (1). Part V has been accepted for publication by *Tetrahedron*.

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

Proton	Compound			
	1	**2**	**3**	**4**
1	5.32 (dd,11.9,2,2)	5.30 (d,10.2)	5.25 (d,10.4)	5.15 (dd,11.9,2.3)
2α	2.09 (m)	—	—	2.11 (dddd,13.0, 13.0,6.0,2.3)
2β	2.41 (dddd,12.5, 12.3,11.9,4.9)	4.70 (ddd,10.3, 10.2,6.1)	4.67 (m)[b]	2.37 (dddd,13.3, 13.0,11.9,5.0)
3α	1.14 (dt,12.5,5.5)	1.23 (dd,11.4,10.3)	1.22 (dd,10.9,10.9)	1.21 (ddd,13.0, 13.0,6.0)
3β	2.15 (br t,12.3)	2.58 (dd,11.4,6.1)	2.56 (dd,12.2,5.9)	2.16 (m)
5	2.55 (d,8.8)	2.73 (d,8.8)	2.77 (d,9.0)	2.69 (d,9.0)
6	3.75 (t,8.7)	3.95 (dd,8.8,8.8)	3.79 (dd,9.0,9.0)	3.80 (dd,9.0,8.4)
7	1.9 (m)	2.38 (ddd,12.1,8.8, 8.6)	2.37 (m)[c]	2.28 (m)
8α	1.9 (m)	—	2.37 (m)[c]	2.28 (m)
8β	1.9 (m)	4.92 (ddd,12.1,8.2, 3.6)	1.60 (m)[c]	1.80 (m)
9α	—	2.44 (m)	1.63 (m)[c]	1.80 (m)
9β	4.11 (dd,7.5,3.9)	2.44 (m)	2.11 (m)[c]	2.25 (m)
11β	2.26 (dq,11.4,7.2)	2.58 (dq,8.6,6.6)	2.30 (dq,10.0,6.9)	2.27 (dq,10.3,6.8)
13	1.24 (d,7.2)	1.45 (d,6.6)	1.30 (d,6.9)[b]	1.25 (d,6.8)
14	1.68 (s)	1.87 (d,1.1)	1.78 (s)	1.68 (s)
15	1.26 (s)	1.30 (s)	1.30 (s)	1.27 (s)
		2.12 (s,OAc)	1.57 (s,OH)	

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

[b]These signals were irradiated during decoupling experiments.

[c]Assignments may be interchanged.

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

Carbon	Compound		
	1	**2**	**4**[b]
1	126.3 (−)	131.0 (−)	125.1 (−)
2	23.8 (+)	66.6 (−)	24.0 (+)
3	36.9 (+)	45.1 (+)	3.6. (+)
4	61.3 (+)	60.6 (+)	61.4 (+)
5	66.1 (−)	66.6 (−)	66.3 (−)
6	81.3 (−)	78.6 (−)	82.1 (−)
7	48.5 (−)	55.4 (−)	51.9 (−)
8	37.8 (+)	72.1 (−)	29.7 (+)
9	79.6 (−)	49.3 (+)	41.1 (+)
10	136.5 (+)	131.0 (+)	134.4 (+)
11	42.1 (−)	39.6 (−)	42.4 (−)
12	176.9 (+)	176.8 (+)	179.6 (+)
13	13.2 (−)	18.3 (−)[c]	13.2 (−)
14	10.9 (−)	18.2 (−)[c]	16.8 (−)[c]
15	17.3 (−)	17.0 (−)	17.1 (−)[c]

[a]Chemical shifts are in ppm from TMS; solvent was $CDCl_3$.

[b]Data taken from Ruangrungsi *et al.* (1).

[c]Assignments may be interchanged.

parthenolide (3) with **1** (a dihydroparthenolide derivative) is helpful. The H-9β resonance at δ 4.34 ppm in the former compound is in the same region as the proposed H-9β in **1** (4.11 ppm), and the coupling constants are also appropriate for a proton at the β position [i.e., a pseudo-equatorial position and, thus, no large coupling with the protons on C-8 (4) assuming the usual conformation in which the C-14 and C-15 methyls are *cis*]. Thus, we propose that component **1** is (−)-9α-hydroxydihydroparthenolide, a new germacranolide.

The ir spectrum of the second component, **2**, exhibited absorptions at 3527 (hydroxyl), 1778 (γ-lactone), and 1735 cm^{-1} (ester). The parent peak at *m/z* 324 was appropriate for the molecular formula $C_{17}H_{24}O_6$, and the fragment at *m/z* 264, $[M-HOAc]^+$, suggested the presence of an acetoxyl group. The ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2), along with the information above, suggested that **2** was a dihydroparthenolide containing hydroxyl and acetoxyl substituents. In the ^{1}H-nmr spectrum, H-1 appears as a sharp doublet because of *trans* coupling with H-2β (J = 10.2 Hz), while in **1** and **4** H-1 is a broad doublet because of coupling with both H-2 protons. Of the two unassigned resonances at 4.92 and 4.70 ppm, the more deshielded proton should be attached to the carbon bearing the acetoxyl group, and, consequently, we propose that the hydroxyl group is at the 2α position, and the H-2β resonance is at 4.70 ppm. Placement of the acetoxyl group at C-8 is consistent with the ^{1}H-nmr spectrum, but, more conclusively, this location results in the anticipated shifts in the ^{13}C-nmr spectrum relative to that of **4**. The C-8 resonance is downfield by 42.4 ppm (α effect; see related discussion for **1**), 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-acetoxyl group is placed in the α position by analogy to the ^{1}H-nmr spectrum of the closely related 8α-acetoxydihydroparthenolide (5). In the latter compound the H-8β resonance appears at 4.90, while in **2** it is found at 4.92 ppm. In 8-acetoxygermacranolides in which H-8 is α, this resonance appears much further downfield at about 5.7 ppm (6). The very significant deshielding of the C-13 methyl group in **2** relative to its position in **4** (18.3 vs. 13.2, respectively) suggests a *syn*-orientation of this methyl and the acetoxyl group (i.e., both groups α). Thus, we propose component **2** is 8α-acetoxy-2α-hydroxydihydroparthenolide, for which we suggest the name paramicholide. For a related discussion of these assignments, see Jakupovic *et al.* (7).

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 (8). Comparison of the spectrum of dihydroparthenolide [**4**] with those of the oxygenated derivatives **1** and **2** enabled us to assign each of these resonances for this family of compounds. Clearly, the resonances at 24.0 and 36.6 ppm in **4** must be assigned to C-2 and C-3 because introduction of a hydroxyl group at C-9 in component **1** has little effect on the chemical shifts of these two carbons. The resonance at 36.6 ppm is assigned to C-3 because of the deshielding effect of the neighboring epoxide function. The resonance at 41.1 ppm in **4** is attributed to C-9 because a methylene carbon adjacent to a methyl group on a trisubstituted *trans* double bond, *trans*-[-CH=C(CH_3)CH_2-], has been shown to be significantly deshielded relative to the other allylic carbon (9). Also, the chemical shifts predicted for these four carbons in **1** and **2** based on the α, β, and γ effects of oxygen substituents on the model system **4** are in excellent agreement with the actual values.

The ir spectrum of the third component, **3**, suggested the presence of a γ-lactone and a hydroxyl group, and the

^{1}H-nmr spectrum again indicated the compound was a substituted dihydroparthenolide (Table 1). The H-1 resonance at 5.25 ppm was a sharp doublet (J = 10.4 Hz) indicating (as discussed previously for **2**) that the hydroxyl group was attached to C-2 and was in the α position. The chemical shift for H-2β is at essentially the same position in both **2** and **3**, suggesting the similarity of structures in this region of the molecules. A ^{13}C spectrum of **3** was not obtained because of the limited amount of sample available and because the sample contained a small amount of **2** as an impurity (relative R_f 0.20 and 0.22). Thus, we conclude that **3** is 2α-hydroxydihydroparthenolide.

To our knowledge, none of these three substituted dihydroparthenolides **1–3** has been isolated previously from natural sources. It is of interest from a biosynthetic perspective that, although the oxygenation of the parent dihydroparthenolide has taken place at three different positions in these constituents, in all cases the substituents were introduced on the α face of the molecule.

EXPERIMENTAL

INSTRUMENTS.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WH-400 spectrometer with TMS (δ = 0) as internal standard. The multiplicities for the ^{13}C spectra were determined by the attached proton test that 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.

PLANT MATERIAL.—The bark of *P. baillonii* was collected and authenticated as previously described (1). A voucher specimen has been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

EXTRACTION AND PURIFICATION.—The fresh bark of *P. baillonii* was extracted with 95% EtOH and the residue purified by Si gel cc first using 70% EtOAc/petroleum ether (bp 30–60°) and then using $CHCl_3$/petroleum ether and $CHCl_3$/Me_2CO mixtures as previously described (1). Fractions 49 and 50 from this latter purification (1) gave 97 mg of an oil which was shown by ^{1}H nmr to be a 2:1:1 mixture of components **1**, **2**, and **3**, respectively. This mixture (50 mg) was separated by flash chromatography using the solvent system MeOH-EtOAc-petroleum ether (10:20:70) to yield 20 mg of **1** and 25 mg of a mixture of **2** and **3**. The latter mixture was separated by flash chromatography using the same solvent system to give 5 mg of **2** and 3 mg of **3** plus several fractions that were still mixtures of **2** and **3** (R_f 0.22 and 0.20, respectively).

(−)-9α-HYDROXYDIHYDROPARTHENOLIDE [**1**].—Tlc MeOH-EtOAc-petroleum ether (10:20:70) R_f 0.32; $[\alpha]^{22}D$ −60° ($CHCl_3$); ir ν max ($CHCl_3$) 3606, 3472 (br), 3550–3400, 2937, 1777, 992 cm^{-1}; ^{1}H and ^{13}C nmr see Tables 1 and 2, respectively; eims *m/z* (rel. int.) $[M]^+$ 266 (5), 249 (9), 248 (9), 208 (28), 190 (29), 135 (64); hrms *m/z* 266.1527 $[M]^+$ (calcd for $C_{15}H_{22}O_4$, 266.1518).

8α-ACETOXY-2α-HYDROXYDIHYDROPARTHENOLIDE (PARAMICHOLIDE) [**2**].—Tlc MeOH-EtOAc-petroleum ether (10:20:70) R_f 0.22; ir ν max ($CHCl_3$) 3527, 3400–3200, 2935, 1778, 1735, 1645, 1235, 1020 cm^{-1}; ^{1}H and ^{13}C nmr see Tables 1 and 2, respectively; eims *m/z* (rel. int.) $[M]^+$ 324 (2), $[M-HOAc]^+$ 264 (3), 249 (7), 155 (45).

2α-HYDROXYDIHYDROPARTHENOLIDE [**3**].—Tlc MeOH-EtOAc-petroleum ether (10:20:70) R_f 0.20; ir ν max ($CHCl_3$) 3600, 3400–3200, 2931, 1774, 1008, 909 cm^{-1}; ^{1}H nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 266 (1).

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