Two New Macrolide Sesquiterpene Pyridine Alkaloids from Maytenus emarginata: Emarginatine G and the Cytotoxic Emarginatine F

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TWO NEW MACROLIDE SESQUITERPENE PYRIDINE ALKALOIDS FROM *MAYTENUS EMARGINATA*: EMARGINATINE G AND THE CYTOTOXIC EMARGINATINE F

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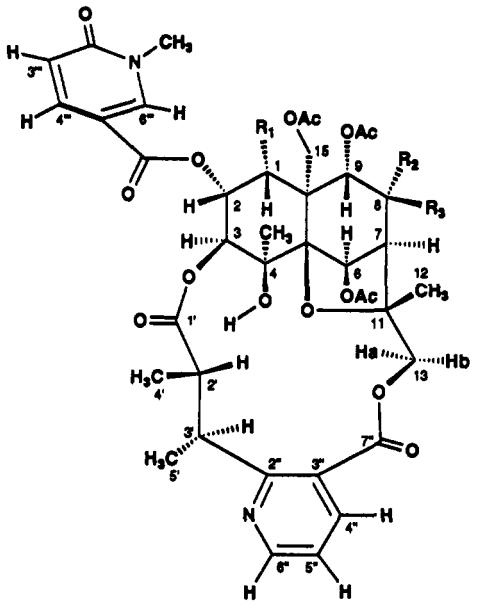
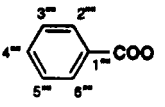
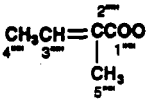
ABSTRACT.—Two new macrolide sesquiterpene pyridine alkaloids, emarginatine F [**1**] and emarginatine G [**2**], were isolated from *Maytenus emarginata*. The structural determinations of **1** and **2** by 2D nmr techniques and spectral comparison with a related compound, emarginatine A [**3**], are discussed. Biological evaluation showed that emarginatine F [**1**] demonstrated strong cytotoxicity against human epidermoid carcinoma of the nasopharynx (KB), ileocecal adenocarcinoma (HCT-8), melanoma (RPMI-7951) and medulloblastoma (TE-671) tumor cells, and against murine leukemia (P-388).

Recently, we reported the isolation and structural elucidation of six new sesquiterpene pyridine alkaloids from *Maytenus emarginata* (Willd.) Hou (Celastraceae) (1–3). Several of these compounds showed cytotoxicity against human KB cells (3). Further investigation of this plant has led to the isolation of two new macrolide sesquiterpene pyridine alkaloids, emarginatine F [**1**] and emarginatine G [**2**], and a known structurally similar compound, hippocrateine. The structures of the new compounds were established by spectral methods, including ^{1}H-^{1}H COSY, NOESY, and ^{1}H-^{13}C long-range COSY studies to assign the ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2), and by comparison with a related compound, emarginatine A [**3**], whose structure was previously elucidated by spectral and X-ray analyses (1). The cytotoxicity of these new compounds against several human tumor cell lines (KB, A-549, HCT-8, RPMI-7951, and TE-671) and one murine leukemia cell line (P-388) was determined. Emarginatine F [**1**] showed strong cytotoxicity in the KB, HCT-8, P-388, RPMI-7951, and TE-671 cell lines and marginal cytotoxicity in the A-549 tumor cell-line.

RESULTS AND DISCUSSION

Emarginatine F [**1**], $C_{46}H_{50}N_2O_{18}$, displayed a uv absorption at 266 nm (pyridone) and ir absorptions at 3500 (OH), 1740 (ester CO), and 1660 (pyridone CO) cm^{-1}. Its ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) suggested a β-agarofuran skeleton with an evoninate diester bridge. This structural class has been reported by our group and by other laboratories (1–8). In plants of the Celastraceae family, the pyridone at the C-2 position appears to be unique to compounds isolated from *M. emarginata*. Comparison of the ^{1}H- and ^{13}C-nmr spectra of **1** with those of emarginatine A [**3**] showed that the two structures were similar except for the absence of two acetate signals in **1**. Instead, an A_2M_2X proton spin system at δ 7.70 (d), δ 7.49 (t), and δ 7.33 (t) and aromatic carbon



	R_1	R_2	R_3
1		H	OH
2		OAc	H
3	OAc	OAc	H

signals at δ 129.15 (C-1‴″), δ 128.44 (C-2‴″, -6‴″), and δ 129.40 (C-3‴″, -5‴″) were found in **1**. This suggested that one acetate had been replaced with a benzoate group, which was consistent with the presence of an eims fragment ion at *m/z* 105. This benzoate group was located at the C-1 position, since ^{1}H-^{13}C heteronuclear long-range COSY (HMBC) studies revealed a correlation between H-1 (δ 6.02) and the benzoate carbonyl (δ 164.40), and a downfield shift (δΔ +0.35 ppm) was observed for H-1 in the ^{1}H-nmr spectra of **1** as compared with **3**. The second missing acetate group was explained by replacement of the C-8 acetate group in **3** with an OH in **1**. This substitution would result in a large upfield shift of H-8 (δΔ −1.15 ppm) and a downfield shift of H-9 (δΔ +0.33 ppm). As further confirmation, the HMBC spectrum of **1** showed that H-6 (δ 6.46) and H-9 (δ 5.75) were correlated with two carbonyl resonances at δ 170.00 and δ 171.50, respectively, while H-8 (δ 4.39) did not correlate with a carbonyl resonance. These data confirmed that the second acetate group was absent from C-8. This spectrum also assigned a third carbonyl carbon resonance (δ 170.65) to the acetate at C-15. A NOESY nmr spectrum of **1** (Figure 1) confirmed the position of the benzoate at the C-1 position due to an enhancement effect between H-2‴″, H-6‴″ of the benzoate and the 15-OAc methyl protons. Mutual enhancements between H-9, H-15, and H-6 with the respective OAc groups at these positions helped to assign the singlets at δ 1.45, δ 2.20, and δ 2.30 ppm to the 9-OAc, 15-OAc, and 6-OAc methyls, respectively. Protons H-6 and H-8 showed mutual enhancements, while H-8 and H-9 did not. This suggested that the former pair of protons are cis, and the latter pair are trans. The coupling constant

TABLE 1. ^{1}H-Nmr (300 MHz) Data for Emarginatines F [**1**], G [**2**], and A [**3**].[a]

Proton	Compound		
	1	**2**	**3**
H-1	6.02 (d, 4.0)	5.80 (d, 4.30)	5.67 (d, 4.2)
H-2	5.55 (dd, 4.0, 2.3)	5.43 (dd, 4.3, 2.2)	5.48 (dd, 4.2, 2.4)
H-3	4.87 (d, 2.3)	4.79 (d, 2.2)	4.78 (d, 2.4)
H-6	6.46 (s)	7.02 (s)	7.04 (s)
H-7	2.43 (d, 3.2)	2.36 (d, 4.2)	2.38 (d, 4.2)
H-8	4.39 (dd, 3.2, 9.5)	5.53 (dd, 4.2, 5.9)	5.54 (dd, 4.2, 6.1)
H-9	5.75 (d, 9.5)	5.38 (d, 5.9)	5.42 (d, 6.1)
H-13	3.72, 5.97 (ABq, 11.7)	3.69, 5.95 (ABq, 11.5)	3.72, 5.98 (ABq, 11.6)
H-15	4.44, 5.46 (ABq, 13.1)	4.19, 5.58 (ABq, 13.4)	4.16, 5.54 (ABq, 13.5)
H-4″	8.08 (dd, 1.6, 7.5)	8.06 (dd, 1.8, 8.0)	8.06 (dd, 1.8, 7.8)
H-5″	7.26 (dd, 3.9, 7.5)	7.26 (dd, 4.9, 8.0)	7.32 (dd, 4.8, 7.8)
H-6″	8.69 (dd, 1.6, 3.9)	8.68, (dd, 1.8, 4.9)	8.70 (dd, 1.8, 4.8)
H-3‴	6.57 (d, 9.6)	6.57 (d, 9.6)	6.59 (d, 9.6)
H-4‴	7.82 (dd, 2.5, 9.6)	7.87 (dd, 2.6, 9.6)	7.90 (dd, 2.5, 9.6)
H-6‴	8.36 (d, 2.5)	8.43 (d, 2.6)	8.42 (d, 2.5)
H-2′	2.63 (q, 6.9)	2.56 (q, 7.1)	2.57 (q, 6.8)
H-3′	4.66 (q, 7.0)	4.66 (q, 7.0)	4.67 (q, 7.0)
Me-4′	1.24 (d, 7.1)	1.19 (d, 7.0)	1.20 (d, 7.0)
Me-5′	1.40 (d, 7.1)	1.37 (d, 7.0)	1.39 (d, 7.0)
Me-12	1.82 (s)	1.71 (s)	1.71 (s)
Me-14	1.58 (s)	1.52 (s)	1.57 (s)
OAc	1.45 (C-9)[b]	1.80 (C-9)[b]	1.81
OAc	2.20 (C-15)[c]	2.11	1.98
OAc	2.30 (C-6)[c]	2.19	2.18
OAc	—	2.36 (C-6)[c]	2.22
OAc	—	—	2.38
H-2⁗, 6⁗	7.70 (d, 7.5)	—	—
H-3⁗, 5⁗	7.33 (t, 7.5)	—	—
H-4⁗	7.49 (t, 7.5)	—	—
Me-4⁗	—	1.65 (d, 8.5)	—
Me-5⁗	—	1.63 (s)	—
H-3⁗	—	6.52 (q, 8.5)	—

[a]Measured in $CDCl_3$; J in Hz; δ=ppm. Multiplicity of signals: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Data for [**3**] from Kuo *et al.* (1).

[b]Assignments by ^{1}H-^{13}C long-range COSY.

[c]Assignments by NOESY.

between H-8 and H-9 was larger in **1** ($J_{8,9}$=9.5 Hz) than in **3** ($J_{8,9}$=6.1 Hz), and the chemical shift of the C-9 carbon was more downfield (δΔ +5.89 ppm) in **1**. These data agree with an α-orientation of H-8 and a trans-diaxial orientation of H-8 and H-9 in **1**, rather than the β, cis-orientation found in **3**. Thus, the structure of emarginatine F [**1**] was determined unambiguously.

Emarginatine G [**2**] showed a molecular ion at *m/z* 938 consistent with a molecular formula of $C_{46}H_{54}N_2O_{19}$. The ^{1}H- and ^{13}C-nmr spectra of **2** were similar to those of **3** except for the absence of one acetate group. The molecular formula of **2** is consistent with the replacement of an acetate group ($C_2H_3O_2$) with a 2-methyl-2-butenoyl group ($C_5H_7O_2$). Its DEPT and ^{1}H-^{1}H COSY spectra confirmed the unique allylic coupling between the H-3⁗ proton (q, δ 6.52, J=8.5 Hz) and the H-4⁗ methyl protons (d, δ 1.65, J=8.5 Hz). ^{1}H-^{13}C heteronuclear nmr data established the carbon shifts for the C-

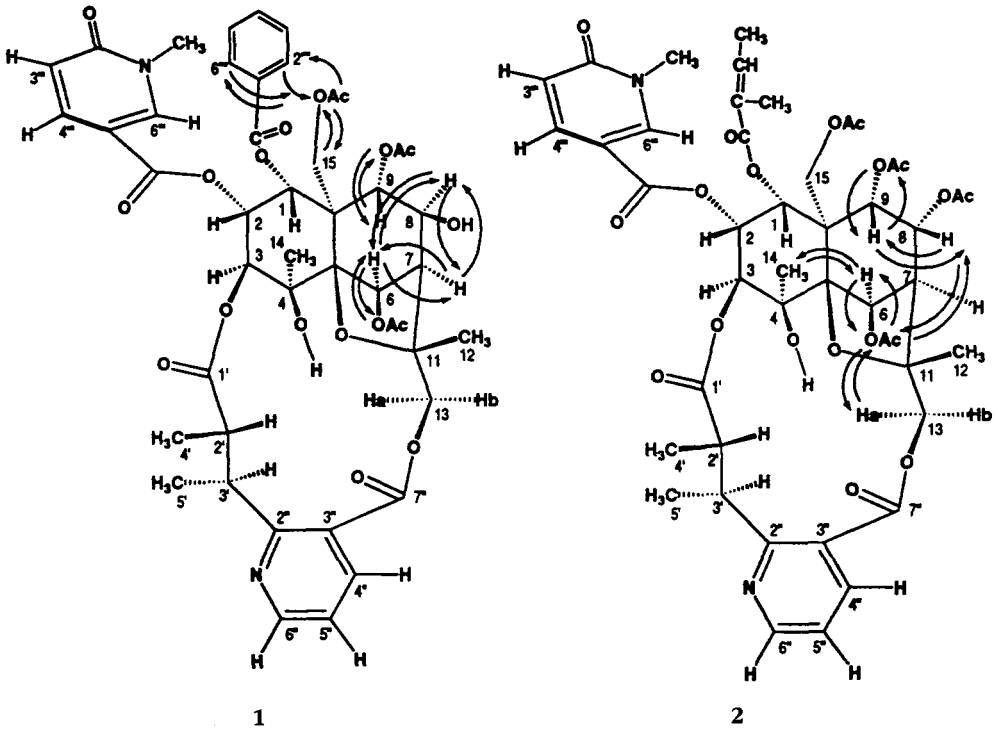
TABLE 2. ^{13}C-Nmr (75.47 MHz) Data[a] for Emarginatine F [**1**], G [**2**], and A [**3**].

Carbon	Compound			^{1}H-^{13}C Connectivities[b]
	1	**2**	**3**	
1	72.20 (d)	72.41 (d)	73.12 (d)	H-3, H-9
2	69.62 (d)	69.67 (d)	69.38 (d)	H-1, H-2, H-3
3	74.90 (d)	75.42 (d)	75.65 (d)	H-14
4	69.95 (s)	70.26 (s)	70.45 (s)	H-2, H-3, H-14
5	94.16 (s)	93.93 (s)	94.09 (s)	H-3, H-6, H-14, H-15
6	74.76 (d)	73.62 (d)	73.75 (d)	H-7, H-8, H-14
7	51.27 (d)	50.51 (d)	50.74 (d)	H-8, H-9, H-12
8	74.38 (d)	68.69 (d)	68.95 (d)	H-6, H-7, H-9
9	76.52 (d)[c]	71.08 (d)	70.63 (d)	H-7, H-8, H-15
10	52.15 (s)	52.26 (s)	52.15 (s)	H-9, H-15
11	84.42 (s)	84.34 (s)	84.42 (s)	H-6, H-7, H-12
12	19.44 (q)	18.48 (q)	18.73 (q)	H-13
13	70.32 (t)	69.89 (t)	70.02 (t)	H-12
14	24.05 (q)	23.16 (q)	23.42 (q)	H-3, 4-OH
15	61.45 (t)	60.40 (t)	60.52 (t)	4-OH
2′	44.76 (d)	44.91 (d)	45.13 (d)	H-3′, H-4′
3′	36.44 (d)	36.42 (d)	36.54 (d)	H-2′, H-4′, H-5′
4′	9.81 (q)	9.73 (q)	9.87 (q)	H-2′, H-3′
5′	12.00 (q)	11.89 (q)	11.99 (q)	H-2′, H-3′
2″	165.10 (s)	165.37 (s)	165.72 (s)	H-2, H-3′, H-4″, H-6″
3″	124.85 (s)	124.95 (s)	125.13 (s)	H-5″
4″	137.53 (d)	137.74 (d)	137.95 (d)	H-6″
5″	120.85 (d)	121.09 (d)	121.31 (d)	H-6″
6″	151.28 (d)	151.54 (d)	151.73 (d)	H-4″
3‴	119.72 (d)	119.85 (d)	120.00 (d)	—
4‴	138.54 (d)	138.92 (d)	139.13 (d)	H-6‴
5‴	107.82 (s)	108.19 (s)	108.35 (s)	H-3‴
6‴	143.55 (d)	143.87 (d)	144.22 (d)	H-4‴, N-CH_3
NCH_3	38.00 (q)	38.12 (q)	38.34 (q)	H-6‴
AcMe	20.45	20.28	20.59	
	21.11	21.03	20.68	
	21.50	21.32	21.24	
	—	21.67	21.49	
	—	—	21.81	
CO-7‴	162.21	162.27	162.65	H-4‴, H-6‴
CO-2‴	162.89	162.90	163.17	H-3‴, H-4‴, H-6, NCH_3
CO-7″	168.51	168.68	168.64	H-4″, H-13a, b
CO-1′	173.87	173.84	174.02	H-2′, H-3′, H-4′, H-3
MeCOO-C-1	—	—	169.01	H-1
MeCOO-C-6	170.00[c]	169.90	170.17	H-6
MeCOO-C-9	171.50[c]	168.51	162.67	H-9
MeCOO-C	170.65[c] (C-15)	171.09[c] (C-15)	170.32	
	—	—	171.18	
BzCO	164.40	—	—	H-1
C-2‴″, 6‴″	128.44	—	—	
C-3‴″, 5‴″	129.40	—	—	
C-4‴″	133.45	—	—	
C-1‴″	129.15	—	—	
R-COO-C-1‴″	—	165.38[c]	—	H-1, H-3‴″
C-2‴″	—	128.01 (s)	—	Me-4‴″, 5‴″
C-3‴″	—	138.12 (d)	—	Me-4‴″, 5‴″
C-4‴″	—	14.37[c] (q)	—	—
C-5‴″	—	11.89[c] (q)	—	H-3‴″

[a]Multiplicities were obtained from DEPT spectra.

[b]^{1}H-^{13}C long range correlation (HMBC) corresponding to 2-bond or 3-bond connectivities. Data for [**3**] from Kuo *et al.* (1).

[c]Assignment of this signal explained in text.



1 2

FIGURE 1. NOESY Correlations in Emarginatines F [**1**] and G [**2**].

4'''' methyl at δ 14.37, the C-3'''' alkene at δ 138.12, and the C-5'''' and C-5' methyls both at δ 11.89. Proper prediction of the carbon resonances at δ 165.38 and δ 128.01 was accomplished by ^{1}H-^{13}C long-range nmr studies. Correlations were found between the carbon resonance at δ 165.38 and H-1 (3-bond) and H-3'''' (3-bond). This assigned the resonance at δ 165.38 to the C-1'''' carbonyl. The carbon resonance at δ 128.01 showed correlations with the H-4'''' (3-bond) and H-5'''' (2-bond) methyl protons. Thus, the resonance at δ 128.01 was assigned to the C-2'''' alkene carbon. A NOESY spectrum (Figure 1) assigned the proton singlets at δ 2.36 and δ 1.80 to the acetate methyls at the C-6 and C-9 positions, respectively, based on mutual enhancements of the former signal with H-6, -8, and -13a and the latter with H-9. Mutual enhancements between H-8 and H-9 also confirmed a cis-relationship of these protons. The structure of emarginatine G [**2**] was thus fully established as 8-*epi*-desacetylhippocrateine 1.

Both emarginatine F [**1**] and G [**2**] were assayed for cytotoxicity against six cancer cell lines, namely, KB, A-549, HCT-8, P-388, RPMI-7951, and TE-671 (Table 3). Emarginatine F [**1**] showed strong cytotoxicity in the KB, HCT-8, P-388, RPMI-7951, and TE-671 cell lines and marginal cytotoxicity in the A-549 tumor cell. Emarginatine

TABLE 3. Cytotoxicity of Emarginatine F [**1**] and G [**2**] Against Tumor Cell Lines.

Compound	Cell Line (ED_{50}, μg/ml)					
	KB	A-549	HCT-8	P-388	TE-671	RPMI-7951
1	0.51	5.50	1.29	0.69	0.21	<0.1
2	I[a]	I[a]	I[a]	I[a]	I[a]	I[a]

[a]I=Inactive, ED_{50}>10 μg/ml.

G [**2**] was inactive in all six cell lines. It has been previously reported that the configuration at C-8 may be important to the cytotoxicity of this compound class (3). Like emarginatine G [**2**], two analogs, emarginatine A [**3**] and emarginatine D (R_1=OH, R_2=OAc, R_3=H), are also H-8 β epimers. The latter compound is also inactive in the KB assay, and the former is only moderately active (ED_{50}=4.09 μg/ml) (3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded at 300 and 75.46 MHz, respectively, on a Bruker 300 AC spectrometer and are reported in ppm relative to TMS as an internal standard. The usual pulse sequences of Bruker were used in ^{1}H-^{1}H COSY, NOESY, and ^{1}H-^{13}C COSY experiments. For the heteronuclear correlations, coupling constants of 8 Hz (long-range) were employed in measurements. Eims were determined on a JEOL SX-102A instrument. Si gel (Merck 70-230 mesh) was used for cc, and precoated Si gel (Merck 60 F-254) plates were used for tlc. Detection of alkaloid compounds was performed with Dragendorff's reagent. Prep. hplc was performed on a Shimadzu SPD-6AV liquid chromatograph using a preparative Nova-Pak C18 column. Mps were determined on a Fisher-Johns apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 983 spectrophotometer and refer to KBr pellets. Uv spectra were measured on a Shimadzu UV-260 spectrophotometer in EtOH.

PLANT MATERIAL.—The stems of *M. emarginata* were collected in January 1992 on Lan Yun Island, Taiwan. A voucher specimen is deposited at the Herbarium of the School of Agriculture, Chinese Culture University, Taipei and at the Herbarium of the National Research Institute of Chinese Medicine, Taipei Hsien, Taiwan, Republic of China.

ISOLATION OF EMARGINATINE F [**1**] AND G [**2**].—The crude $CHCl_3$ fraction (120 g) from the liquid-liquid partition of the MeOH extract reported previously was subjected to cc over Si gel (2 kg). Elution with a $CHCl_3$/MeOH gradient yielded fractions A, B, C, and D. Fraction C was further chromatographed using $CHCl_3$, $CHCl_3$/Me_2CO, and Me_2CO to yield 7 portions. Separation by hplc [MeOH-H_2O (7:3)] gave emarginatine F [**1**] (9 mg) from the third portion, and emarginatine G [**2**] (15 mg) and hippocrateine from the fourth portion.

Emarginatine F [**1**].—White crystalline needles mp 221–223°; $[\alpha]^{20}D$ +600° (*c*=0.01, $CHCl_3$); uv λ max (ε) 266 (12200) nm; ir ν max 3500, 1740, 1660, 1580, 1560, 1440, 1290, 710 cm^{-1}; eims *m/z* $[M]^+$ 918 (27), 859 (11), 706 (8), 650 (7), 530 (27), 218 (29), 206 (64), 136 (100), 105 (95); ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2.

Emarginatine G [**2**].—White crystalline needles mp 315–318°; $[\alpha]^{20}D$ +200° (*c*=0.02, $CHCl_3$); uv λ max (ε) 266 (12200) nm; ir ν max 3500, 1742, 1662, 1580, 1562, 1440, 1290, 710 cm^{-1}; eims *m/z* $[M]^+$ 938 (35), 910 (4), 898 (5), 895 (7), 879 (7), 855 (6), 726 (13), 686 (13), 573 (15), 572 (46), 262 (9), 218 (8), 206 (81), 178 (36), 154 (20), 136 (100), 107 (44), 106 (13), 105 (7); ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2.

BIOLOGICAL ASSAYS.—The in vitro cytotoxicity assay was carried out according to procedures described in Geran *et al.* (9) and Ferguson *et al.* (10). The assay against KB (nasal pharyngeal carcinoma), A-549 (human lung carcinoma), HCT-8 (human colon adenocinoma), RPMI-7951 (human melanoma), TE-671 (human medulloblastoma), and P-388 (murine leukemia) tumor cells was based on a method reported in Lee *et al.* (11).

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