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A NEW TRITERPENE LACTONE, MAYTENFOLONE, AND A NEW SESQUITERPENE PYRIDINE ALKALOID, EMARGINATINE H, FROM THE LEAVES OF *MAYTENUS DIVERSIFOLIA*

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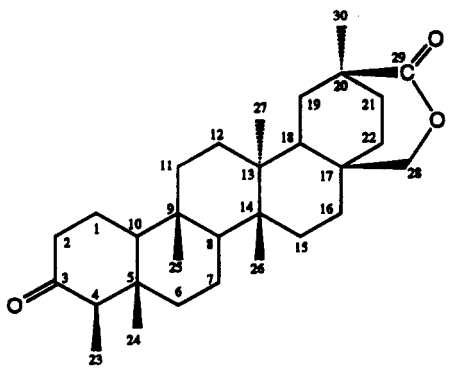
ABSTRACT.—A new triterpene lactone, maytenfolone [**1**], and a new sesquiterpene pyridine alkaloid, emarginatine H [**2**], as well as the known triterpene friedelin, were isolated from the leaves of *Maytenus diversifolia*. The determination of their structures was based on 2D nmr techniques and other spectral data.

Maytenus diversifolia (Gray) Ding Hou [=*Gymnosporia diversifolia* (Gray) Maxim.] (Celastraceae) is known as "Pak-Tiong (Pei-Chung)" or "Tzu-Lou-Shih" in southern Taiwan. Although a MeOH extract of the stems of *M. diversifolia* has been investigated and found to contain antileukemic triterpenes and the antitumor alkaloid maytansine (1–3), the leaves of *M. diversifolia* have not been investigated previously and were considered worthy of study in our continuing search for potential plant antitumor agents.

We describe herein the structural elucidation of a new triterpene lactone, maytenfolone [**1**], and a new agarofuran sesquiterpene alkaloid, emarginatine H [**2**]. The complete assignment of the ^{1}H- and ^{13}C-nmr spectra of **1** and **2** employed 2D nmr techniques, including ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, and ^{1}H-^{13}C long-range COSY (COLOC and HMBC). The structural elucidation of emarginatine H involved data comparison with the known sesquiterpene pyridine alkaloid emarginatine A [**3**]. The latter compound exhibits marginal cytotoxicity in KB cells and was isolated previously by our group from *M. emarginata* (4,5).



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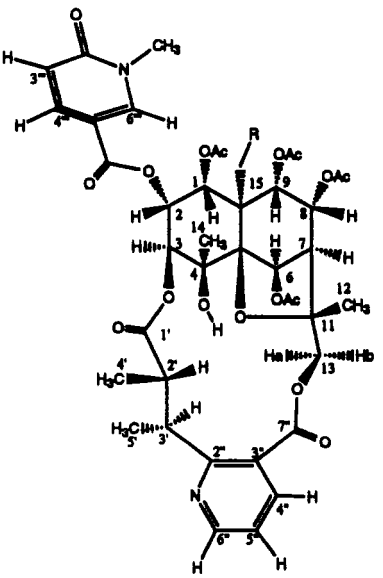

R
2 OH
3 OAc

An EtOH extract of the dried leaves of *M. diversifolia* was extracted successively with *n*-hexane and $CHCl_3$. Chromatographic fractionation of the $CHCl_3$ extract gave compounds **1**, **2**, and friedelin.

Compound **1** has the molecular formula $C_{30}H_{46}O_3$. One secondary and five tertiary methyl groups were found in the ^1H- and ^{13}C DEPT nmr spectra; the presence of signals at δ_C 213.13 (s) and 1715 cm^{-1} in the ^{13}C-nmr and ir spectra, respectively, indicated one ketone. A characteristic peak at *m/z* 273 in the mass spectrum revealed fragmentation leading to the A, B, and C rings in a manner characteristic of friedelin. These data therefore suggested that **1** was an analogue of friedelin except for the absence of two methyl groups and the presence of two doublet protons at δ 3.95 and 4.02. Although the two doublet signals are typical of a CH_2OH group, this functionality was excluded from the structure of **1** because the corresponding carbon signal at δ_C 83.50 is about 10 ppm higher than those typical of CH_2OH groups in triterpenes (2,3,6), and no acetate was obtained after attempted acetylation of **1**. In addition, a carboxylic acid carbon signal at δ 177.95, an ir absorption at 1705 cm^{-1}, and the molecular ion at *m/z* 454 suggested the presence of a lactone containing a methylene group between C-17 and C-20 in compound **1**.

To further deduce the structure and all chemical shifts of **1**, a comparison between the ^{13}C- (DEPT) and ^1H-nmr spectra of **1** and friedelin (9,10) was made. Data from the ^1H-^1H COSY, ^1H-^{13}C COSY, and long-range COSY spectra (HMBC and COLOC) of **1** were also used. From these data, the chemical shifts of carbons and protons from C-1 to C-10 were found to be nearly identical for **1** and friedelin. From the COLOC spectrum, the signals of the high-field methyl groups were correlated with long-range carbons and confirmed their respective chemical shifts. Inspection of the HMBC spectrum of **1** showed that most of the carbon signals from C-11 to C-22 had good long-range correlations with their respective methyl groups, except for C-16 and C-17. The carbon signal at 34.64 ppm was correlated with the proton signals for H-18 (1.96 ppm) and H-28 (3.97 and 3.89 ppm, ABq) and was assigned to C-17, whereas the long-range correlations between C-28 and H-16 placed the chemical shifts of H-16 at δ 1.31 and 1.43 ppm. This led to the observation of the correlation between the C-16 and H-16 positions in the ^1H-^{13}C COSY nmr spectrum, exhibiting the value of δ_C 29.54 for C-16. Moreover, due to the coupling between δ_H 1.75 (H-19) and δ_H 1.96 (H-18) in the ^1H-^1H COSY spectrum, the carbon signal at δ 33.20 was assigned as C-19 and was therefore distinguished from the carbon signals at 33.20, 30.03, and 41.38 ppm, which were correlated with the methyl group at C-20 (δ_H 1.18). The further correlations between C-29 at δ_C 177.95 and the proton signals of H-19, 21, 22, and 28, as well as with the methyl group at C-30 , are also consistent with the lactone of **1** being composed of a seven-membered ring, C-17–C-18–C-19–C-20–C-29–O–C-28. In addition, comparison of the ^{13}C-nmr spectra of **1** and friedelin showed changes in the chemical shifts consistent with the proposed structure of **1**. The absence of one methyl group and the addition of a CH_2O at C-17 linking to the CO at C-20 would induce an increase in the chemical shifts of C-17 (δ_C 34.65 in **1**; δ_C 29.88 in friedelin) and C-22 (δ_C 41.38; δ_C 39.14 in friedelin), as well as a decrease in the chemical shifts of C-16 (δ_C 29.54; δ_C 35.96 in friedelin) and C-18 (δ_C 39.47; δ_C 42.61 in friedelin). The presence of the lactone carbonyl carbon at C-20 would induce an increase in the chemical shift of C-20 (δ_C 33.32; δ_C 28.07 in friedelin) due to the deshielding effect produced by the lactone. Together the above evidence completely confirmed the structure of **1**.

Compound **2** has the molecular for-

mula $C_{14}H_{48}N_2O_{18}$ (*m/z* 856 $[M]^+$). It showed ir absorptions at 3400 (OH) and 1740 (ester) cm^{-1}. From the ^1H-nmr data (Table 1) and its ^1H-^1H COSY spectrum, four acetyl (δ 1.44–2.36), two methyl (δ 1.72, s, H-12; 1.54, s, H-14), two methylene (δ 3.70 and 5.97, AX, J=11.6 Hz, H-13a, -13b; δ 4.31, 5.68, AX, J=13.3 Hz, H-15a, -15b), seven methine protons [AMX system for H-1, H-2, and H-3 at δ 5.97, 5.50, and 4.83 (J=4.2 and 2.4 Hz) and for H-7, H-8, and H-9 at δ 2.40, 5.46, and 5.56 (J=4.1 and 6.0 Hz); δ 7.04, s, OH-6] were detected. This suggested that **2** is an agarofuran-type sesquiterpene. In addition, the signals of a 2,3-disubstituted pyridine unit were found in the aromatic region, and the coupling between the two methyl (δ 1.21 and 1.38) and two methine (δ 2.59 and 4.67) protons revealed a macrocyclic diester linkage at C-3 and C-13 between the evoninate bridge and the sesquiterpene ring system. Moreover, in the ^1H-^{13}C long-range COSY spectrum (HMBC) of **2** (Table 2), the correlation between C-6‴ (δ_C 144.09), C-2‴ (δ_C 163.28), and the *N*-methyl group (δ_H 3.72) indicated that the *N*-methyl group in the pyridonyl moiety is near the C-6‴ and 2‴ positions. Analysis of the above data suggested that **2** is a pyridine sesquiterpene with the characteristic pyridonyl group of compounds obtained from Celastraceae plants (4,5,7,8). The nmr spectra of **2** are comparable with those of **3** (*m/z* 898 $[M]^+$), the known sesquiterpene alkaloid emarginatine A, except for the absence of one acetyl methyl group and the downfield shifts of the H-15 and H-1 protons. Fur-

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

Proton	Compound	
	2	**3**
H-1	5.97 (d, 4.2)	5.67 (d, 4.2)
H-2	5.50 (dd, 4.2, 2.4)	5.48 (dd, 4.2, 2.4)
H-3	4.83 (d, 2.3)	4.78 (s)
H-6	7.04 (s)	7.04 (s)
H-7	2.40 (d, 4.1)	2.38 (d, 4.2)
H-8	5.46 (dd, 4.1, 6.0)	5.54 (dd, 4.2, 6.1)
H-9	5.56 (d, 6.0)	5.42 (d, 6.1)
H-13	3.70, 5.97 (ABq, 11.6)	3.72, 5.98 (ABq, 11.6)
H-15	4.31, 5.68 (ABq, 13.3)	4.16, 5.54 (ABq, 13.5)
H-4″	8.06 (dd, 1.7, 7.8)	8.06 (dd, 1.8, 7.8)
H-5″	7.30 (dd, 1.6, 4.8)	7.32 (dd, 4.8, 7.8)
H-6″	8.69 (dd, 1.6, 4.8)	8.70 (dd, 1.8, 4.8)
H-3‴	6.58 (d, 9.6)	6.59 (d, 9.6)
H-4‴	7.79 (dd, 2.5, 9.6)	7.90 (dd, 2.5, 9.6)
H-6‴	8.42 (d, 2.5)	8.42 (d, 2.5)
H-2′	2.59 (q, 6.9)	2.57 (q, 6.8)
H-3′	4.67 (q, 7.0)	4.67 (q, 7.0)
Me-4′	1.21 (d, 7.0)	1.20 (d, 7.0)
Me-5′	1.38 (d, 7.0)	1.39 (d, 7.0)
Me-12	1.72 (s)	1.71 (s)
Me-14	1.54 (s)	1.57 (s)
OAc	1.44	1.81
OAc-15	—	1.98[b]
OAc	2.13	2.18
OAc	2.20	2.22
OAc	2.36	2.38

[a]Measured in $CDCl_3$.
[b]Assignment of this signal explained in text.

TABLE 2. ^{13}C-Nmr (75.47 MHz) Data[a] for Emarginatines H [**2**] and A [**3**].

Carbon	Compound		^{1}H-^{13}C Connectivities[b]
	2	**3**	
1	73.54 (d)	73.12 (d)	H-3, H-9
2	68.63 (d)	69.38 (d)	H-1, H-2, H-3
3	75.40 (d)	75.65 (d)	H-14
4	69.85 (d)	70.45 (s)	H-2, H-3, H-14
5	93.95 (s)	94.09 (s)	H-3, H-6, H-14, H-15
6	73.53 (d)	73.75 (d)	H-7, H-8, H-14
7	50.50 (d)	50.74 (d)	H-8, H-9, H-12
8	70.84 (d)	68.95 (d)	H-6, H-7, H-9
9	69.43 (d)	70.63 (d)	H-7, H-8, H-15
10	52.35 (s)	52.15 (s)	H-9, H-15
11	84.44 (s)	84.42 (s)	H-6, H-7, H-12
12	18.48 (q)	18.73 (q)	H-13
13	70.31 (t)	70.02 (t)	H-12
14	23.23 (q)	23.42 (q)	H-3, OH-4
15	60.18 (t)	60.52 (t)	H-1, H-9, AcMe-15
2′	44.97 (d)	45.13 (d)	H-3′, H-4′
3′	36.40 (d)	36.54 (d)	H-2′, H-4′, H-5′
4′	9.76 (q)	9.87 (q)	H-2′, H-3′
5′	11.82 (q)	11.99 (q)	H-2′, H-3′
2″	165.34 (s)	165.72 (s)	H-2, H-3′, H-4″, H-6″
3″	124.92 (s)	125.13 (s)	H-5″
4″	137.77 (d)	137.95 (d)	H-6″
5″	121.13 (d)	121.31 (d)	H-6″
6″	151.57 (d)	151.73 (d)	H-4″
3‴	119.94 (d)	120.00 (d)	—
4‴	138.75 (d)	139.13 (d)	H-6‴
5‴	107.83 (s)	108.35 (s)	H-3‴
6‴	144.09 (d)	144.22 (d)	H-4‴, N-CH_3
NCH_3	38.20 (q)	38.34 (q)	H-6‴
AcMe	20.00	20.59	
	21.00	20.68	
	21.27	21.24	
	21.60	21.49	
CO-7‴	162.45	162.65	H-4‴, H-6‴
CO-2″	163.28	163.17	H-3‴, H-4‴, H-6, NCH_3
CO-7′	168.50	168.64	H-4″, H-13a,b
CO-1′	173.82	174.02	H-2′, H-3′, H-4′, H-3
MeCOO-C	168.83 (C-8)[b]	169.01	
MeCOO-C	169.90 (C-6)[b]	170.17	
MeCOO-C	162.93	162.67	
MeCOO-C	170.93	170.32	
MeCOO-C	—	171.18	

[a]Multiplicities were obtained from DEPT spectra; measured in $CDCl_3$.

ther, the mol wt of **2** is 42 units (C_2H_2O) lower than that of **3**, consistent with the substitution of an OH for an OAc group; this change might be in accordance with the downfield shift caused by the loss of the shielding effect of an acetate.

A comparison of the ^{1}H-nmr spectra of **2** and **3** (Table 2) showed not only that the protons at H-15a (δ 4.31 in **2**; δ 4.16 in **3**) and at H-1 (δ 5.97 ppm in **2**; δ 5.67 in **3**) were shifted downfield, but also that one of the acetyl group signals (δ 1.98 ppm) was missing from **2**. No obvious differences of the other proton chemical

shifts were found between **2** and **3**; this suggested that a hydroxy group replaced the acetate group at either the C-15 or the C-1 position. If an OH group replaced the OAc group at the C-1 position, the H-1 signal would be shifted to higher field due to the reduced deshielding effect, and the reported compound emarginatine C (5) would be obtained. Moreover, after a detailed inspection of the HMBC spectrum of **3**, the carbon at δ 60.52 was correlated with the proton shifts at δ 4.16, 5.54 (H-15a,b) and δ 1.98, confirming the presence of an acetate methyl at C-15. Thus, the above results unambiguously established the structure of compound **2** as an analogue of compound **3**, where the acetyl group at C-15 in **3** was replaced by an OH group in compound **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were recorded on a Fisher-Johns apparatus and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were obtained at 300.13 and 75.47 MHz, respectively, on a Bruker AC-300 spectrometer. Cc was performed on Si gel 70–230 or 230–400 mesh (Merck) and tlc on precoated Si gel 60 F_{254} plates (Merck). The alkaloid was detected by spraying with Dragendorff's reagent.

PLANT MATERIAL.—The leaves of *M. diversifolia* were collected in June 1992, in southern Taiwan. A voucher specimen is deposited at the Herbarium of the National Research Institute of Chinese Medicine, Taipei Hsien, Taiwan, Republic of China.

EXTRACTION AND ISOLATION.—The dried leaves of *M. diversifolia* (5 kg) were extracted exhaustively with EtOH. Removal of solvent *in vacuo* gave a syrup, which was extracted successively with hexane and $CHCl_3$-H_2O (3:2). The $CHCl_3$ extract (100 g) was subjected to cc on Si gel (2 kg). Elution with the $CHCl_3$/MeOH gradient yielded 5 fractions. Fraction 2 was rechromatographed to afford 10 portions, and portion 2 gave triterpenes including maytenfolone [**1**] and friedelin. Portion 3 was further purified by hplc (Nucleosil 7C18 MeOH-H_2O, 7:2, 3 ml/min) to yield emarginatine H [**3**].

Maytenfolone [**1**].—White amorphous powder, mp 276–279°; [α]D −84° (*c*=0.1, $CHCl_3$); ir ν max 2980, 2940, 2910, 1715, 1390, 1190 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.97, 3.89 (2H, ABq, *J*=11.5 Hz, H-28), 2.35, 2.22 (2H, m, H-2), 2.23 (1H, m, H-4), 2.12, 1.42 (2H, m, H-22), 1.96 (1H, m, H-18), 1.93, 1.63 (2H, m, H-1), 1.93, 1.48 (2H, m, H-21), 1.75, 1.47 (2H, m, H-19), 1.72, 1.22 (2H, m, H-6), 1.52 (1H, m, H-10), 1.46, 1.27 (2H, m, H-7), 1.46, 1.25 (2H, m, H-15), 1.45, 1.32 (2H, m, H-12), 1.44, 1.25 (2H, m, H-11), 1.43, 1.31 (2H, m, H-16), 1.38 (1H, m, H-8), 1.18 (1H, s, H-30), 1.08 (1H, s, H-27), 0.86 (3H, s, H-25), 0.84 (3H, d, *J*=4.8 Hz, H-23), 0.83 (3H, s, H-26), 0.69 (3H, s, H-24); ^{13}C nmr ($CDCl_3$) δ 212.80 (s, C-3), 177.95 (s, C-29), 83.80 (t, C-28), 59.36 (d, C-10), 58.12 (d, C-4), 50.70 (d, C-8), 41.91 (s, C-5), 41.38 (t, C-2 and C-22), 41.01 (t, C-6), 39.47 (d, C-18), 38.97 (s, C-13), 38.47 (s, C-14), 37.71 (s, C-9), 34.72 (t, C-11), 34.64 (s, C-17), 33.32 (s, C-20), 33.20 (t, C-19), 30.03 (t, C-21), 29.54 (t, C-16), 28.98 (t, C-15), 28.28 (t, C-12), 27.85 (q, C-30), 22.22 (t, C-1), 18.09 (q, C-25), 18.08 (t, C-7), 16.63 (q, C-26), 15.23 (q, C-27), 14.59 (q, C-24), 6.77 (q, C-23); hrms *m/z* [M]$^+$ 454 (50), 369 (100), 273 (21), 149 (28), 109 (51), 95 (52), 81 (49); found *m/z* 454.3443, $C_{30}H_{46}O_3$ requires [M]$^+$ 454.3449.

Friedelin.—Mp 258–260°; [α]D −13.1° (*c*=0.05, $CHCl_3$); eims *m/z* [M]$^+$ 440; this compound was identified by comparison with an authentic sample (9,10).

Emarginatine H [**2**].—White amorphous powder; mp 313–316°; [α]D +35° (*c*=0.05, $CHCl_3$); ir ν max 3500, 1740, 1660, 1580, 1440, 1365, 1284, 710 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1; eims *m/z* [M]$^+$ 856 (10), 842 (11), 809 (12), 749 (14), 572 (30), 206 (54), 136 (100).

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