Journal of Natural Products
Vol. 58, No. 11, pp. 1735-1738, November 1995

CELAHININE A, A NEW SESQUITERPENE PYRIDINE ALKALOID FROM *CELASTRUS HINDSII*

YAO-HAUR KUO,* CHIEH-FU CHEN, LI-MING YANG KUO,

National Research Institute of Chinese Medicine, Taipei Hsien, 23177, Taiwan, Republic of China

MING-LU KING, CHIA-FU CHEN,

School of Pharmacy, National Defense Medical Center, Taipei, Taiwan, Republic of China

and KUO-HSIUNG LEE

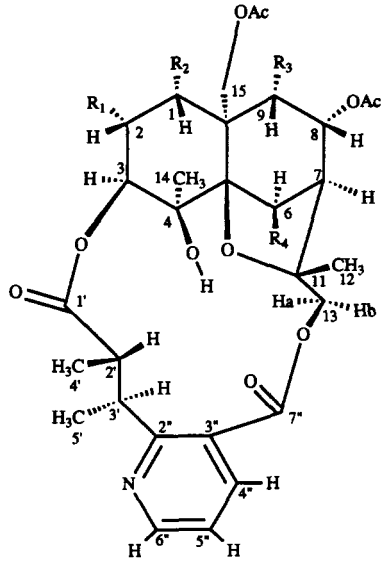
Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599

ABSTRACT.—A new sesquiterpene pyridine alkaloid, celahinine A [**1**], and the related known polyester celahin A, as well as the known cytotoxic sesquiterpene pyridine alkaloid emarginatine A [**2**], were isolated from *Celastrus hindsii*. The structure of **1** was determined by 2D nmr techniques and was also confirmed by spectral comparison with the related **2**.

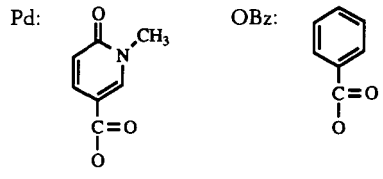
In our search for potential antitumor agents from the family Celastraceae, we recently reported the isolation of new cytotoxic sesquiterpene pyridine alkaloids from the plant *Maytenus emarginata* (Gray) Hou (1–4). We now report that the EtOH extract of *Celastrus hindsii* Benth. shows potent cytotoxicity against Hepa-2 (hepatoma), Hela (cervix carcinoma), Colo 205 (colon carcinoma), and KB (nasopharynx carcinoma) cells in vitro. A new sesquiterpene pyridine alkaloid with a β-dihydroagarofuran skeleton, celahinine A [**1**], a related sesquiterpene polyester, celahin A, and a known cytotoxic sesquiterpene pyridine alkaloid, emarginatine A [**2**, ED_{50} (KB)=4.0 μg/ml] (1) were isolated from this extract. The structure of the new compound **1** was assigned mainly from ^{1}H- and ^{13}C-nmr spectra by employing 2D nmr techniques including ^{1}H-^{1}H COSY, NOESY, and ^{1}H-^{13}C heteronuclear COSY as well as long-range COSY (HMBC and COLOC) experiments.

The EtOH extract of the dried stems of *C. hindsii* was extracted successively with *n*-hexane and $CHCl_3$. Repeated cc of the $CHCl_3$ extract yielded the novel sesquiterpene pyridine alkaloid [**1**] and the known sesquiterpene polyester celahin A.

Compound **1** analyzed for $C_{53}H_{53}NO_{18}$.



	R_1	R_2	R_3	R_4
1	OAc	OBz	OBz	OBz
2	Pd	OAc	OAc	OAc



The ir spectra showed absorption bands at 3400 (OH), 1740 (ester CO), and 1600 and 1450 (benzoate) cm^{-1}. The ^{1}H-nmr spectrum confirmed that compound **1** con-

tained three A_2M_2X proton spin-systems (Bz1: 8.30, 8.06, 7.48; Bz2: 8.30, 8.07, 7.48; Bz3: 7.66, 7.58, 7.28 ppm), one pyridine ring (8.70, 8.06, 7.25 ppm), three acetate groups, and four methyl groups. The ^{13}C-nmr spectrum showed three benzoate carbonyl carbons (164.71–165.81 ppm) and five ester carbonyl carbons (164.62–173.84 ppm). From the ^{1}H-^{1}H COSY nmr spectrum, the signal at δ 7.19 was assigned as H-6ax because, as is common in this type of compound, this proton was only weakly coupled to the high-field signal, H-7eq (δ 2.55) (5). In addition, the two methyl groups at δ 1.24 and 1.44 ppm were obviously coupled to H-2′ (δ 2.65) and H-3′ (δ 4.73), respectively; these data as well as other methine coupling correlations suggested that **1** has a β-agarofuran skeleton with an evoninate diester bridge. This type of structure has been reported previously by our group (1–3).

Comparison of the ^{1}H- and ^{13}C-nmr spectra of **1** (Tables 1 and 2) with those of its analogue, emarginatine A [**2**], which was also isolated from stems of *C. hindsii* and from *M. emarginata* (1), showed that the two structures were similar except that the pyridone and three acetates in **2** had been replaced by one acetate and three benzoates, respectively, in **1**. The appropriate assignment of the position of the benzoates required the use of ^{1}H-^{13}C heteronuclear nmr studies. In the ^{1}H-^{13}C long-range COSY nmr spectrum (HMBC) of **1**, the chemical shifts of both H-6 (δ 7.19) and an aromatic proton at δ 8.30 correlated with the benzoate carbonyl carbon at δ 165.81 ppm, suggesting that one benzoate group was located at C-6. A

TABLE 1. ^{1}H-Nmr (300 MHz) Data for Celahinine A [**1**] and Emarginatine A [**2**].[a]

Proton(s)	Compound	
	1	**2**
H-1	6.08 (d, 4.2)	5.67 (d, 4.2)
H-2	5.65 (dd, 4.2, 2.3)	5.48 (dd, 4.2, 2.4)
H-3a	4.94 (d, 2.3)	4.78 (d, 2.4)
H-6	7.19 (s)	7.04 (s)
H-7	2.55 (d, 3.9)	2.38 (d, 4.2)
H-8	5.57 (dd, 4.2, 5.8)	5.54 (dd, 4.2, 6.1)
H-9	5.50 (d, 5.8)	5.42 (d, 6.1)
H-13	3.64, 6.05 (ABq, 11.8)	3.72, 5.98 (ABq, 11.6)
H-15	4.65, 5.67 (ABq, 13.3)	4.16, 5.54 (ABq, 13.5)
Me-12	1.76 (s)	1.71 (s)
Me-14	1.67 (s)	1.57 (s)
H-2′	2.65 (q, 6.8)	2.57 (q, 6.8)
H-3′	4.73 (q, 7.0)	4.67 (q, 7.0)
Me-4′	1.24 (d, 6.8)	1.20 (d, 7.0)
Me-5′	1.44 (d, 6.8)	1.39 (d, 7.0)
H-4″	8.06 (m)	8.06 (dd, 1.8, 7.8)
H-5″	7.26 (m)	7.32 (dd, 4.8, 7.8)
H-6″	8.70 (dd, 1.6, 4.8)	8.70 (dd, 1.8, 4.8)
OAc	1.25	1.81
OAc	1.34	1.98
OAc	2.19 (C-15)[b]	2.18
OAc	2.29	2.22
OAc	—	2.38
Bz: H-2, H-6	8.30 (m, ×2), 7.66 (m)	—
Bz: H-3, H-5	7.48 (m, ×2), 7.28 (m)	—
Bz: H-4	8.07 (m, ×2), 7.58 (m)	—

[a]Measured in $CDCl_3$; data for **2** from Kuo *et al.* (1)
[b]Assignments by ^{1}H-^{13}C long-range COSY nmr.

detailed inspection of the HMBC spectrum revealed the correlation of the carbonyl carbon at δ 164.74 with signals at δ_H 8.07 (aromatic proton) and 6.08 ppm (H-1), suggesting that an acetate group at C-1 had been replaced by a benzoate group. Further comparison of the ^{1}H-nmr spectra of **1** and **2** showed that the chemical shifts of H-1 (δ 5.67) and H-15a/H-15b (δ 4.16, 5.54) in compound **2** had been shifted downfield to δ 6.08 (H-1) and δ 4.64 and 5.67 (H-15a/H-15b) in compound **1**. This shift is consistent with the replacement of the acetate at C-1 with a benzoate group. A similar substitution and downfield shift occurred between **2** and emarginatine F as reported previously (4). Moreover, in the HMBC spectrum, the carbonyl carbon at δ 168.89 coupled with signals at δ_H 8.07 (aromatic proton) and at δ_H 5.50 ppm (H-9), suggesting that the remaining benzoate group replaced an acetate group at C-9. The position of the final ester substitution could be predicted from the nmr and mass spectra. Thus, the methyl pyridone fragment at *m/z* 136 from the C-2 position of **3** was replaced and compensated for by an acetate group of *m/z* 59, consistent with the molecular ion of compound **1** at *m/z* 991. Therefore, with the above corroborations, the structure of celahinine A [**1**] has been established as shown.

Celahin A was obtained as an amorphous powder. It was identified by comparison of its physical and spectral data with those reported in the literature for this compound (5). As stated in previous reports (5–8), the stereochemistry of H-1 and H-6 in β-agarofuran compounds are generally axial, and these stereochemistries are found in celahin A and in **1** and **2**. Likewise, the configuration of the oxido bridge is β-axial (9,10).

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

Carbon	Compound	
	1	**2**
1	73.3 (d)	73.1 (d)
2	69.8 (d)	69.4 (d)
3	75.8 (d)	75.7 (d)
4	70.6 (s)	70.5 (s)
5	93.8 (s)	94.1 (s)
6	74.8 (d)	73.8 (d)
7	50.4 (d)	50.7 (d)
8	69.1 (d)	69.0 (d)
9	71.5 (d)	70.6 (d)
10	52.6 (s)	52.2 (s)
11	84.5 (s)	84.4 (s)
12	18.4 (q)	18.7 (q)
13	70.0 (t)	70.0 (t)
14	23.3 (q)	23.4 (q)
15	60.7 (t)	60.5 (t)
2′	45.0 (d)	45.1 (d)
3′	36.5 (d)	36.5 (d)
4′	9.8 (q)	9.9 (q)
5′	11.9 (q)	12.0 (q)
2″	165.3 (s)	165.7 (s)
3″	125.2 (s)	125.1 (s)
4″	137.7 (d)	138.0 (d)
5″	121.1 (d)	121.3 (d)
6″	151.5 (d)	151.7 (d)
AcMe	19.8 (q)	20.6 (q)
	21.1	20.7
	21.3	21.2
	—	21.5
	—	21.0
CO-1′	173.8	174.0
CO-7″	168.6	168.6
MeCOO-C	164.6	169.1
McCOO-C	170.1	170.2
MeCOO-C	170.4	162.7
MeCOO-C	—	170.3
MeCOO-C	—	171.2
BzCO	168.9 (C-9)[b]	—
	164.7 (C-1)[b]	—
	165.8 (C-6)[b]	—
Bz: C-2,6	128.7, 128.4, 128.8	—
C-3,5	129.5, 129.6, 129.9	—
C-4	133.3, 133.5, 133.5	—
C-1	129.1, 129.5, 129.6	—

[a]Multiplicities were obtained from DEPT spectra.

[b]Assignments of this signal explained in the text.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded at 300.13 and 75.46 MHz, respectively, on a Bruker AC 300 spectrometer. The heteronuclear correlation spectra, HMBC and COLOC, were established using coupling constants of 8 Hz. Eims were carried out on a JEOL SX-102A instrument. Si gel (Merck 70–230 mesh) was used for cc, and precoated Si gel (Merck 60F-254) plates were used for tlc. Hplc

was performed on a SPD-6AV liquid chromatograph using a prep. Si gel column. Mps were determined on a Fisher-Johns apparatus and are reported uncorrected.

PLANT MATERIAL.—The stems of *Celastrus hindsii* were collected in September 1992, in Taishung Hsien, Taiwan. A voucher specimen is deposited at the National Research Institute of Chinese Medicine, Taipei Hsien, Taiwan, Republic of China.

EXTRACTION AND ISOLATION.—The dried stems of *C. hindsii* (5.2 kg) were extracted exhaustively with EtOH. The crude EtOH extract (200 g) was chromatographed on Si gel (2.5 kg) eluting with hexane/EtOAc and EtOAc to yield 8 portions. The bioactive portions 3 and 4 were further separated by repeated hplc (Si gel, hexane-EtOAc, 1:3) to yield celahinine A [**1**] (6 mg, 0.000095% yield) and emarginatine A [**2**] (4 mg, 0.000063% yield) from portion 3, and celahin A (20 mg, 0.00032% yield) from portion 4.

Celahinine A [**1**].—Amorphous, mp >300°; ir (KBr) ν max 3500, 1740, 1600, 1450, 1280, 710 cm^{-1}; eims *m/z* 993 (1), 992 (4) $[M]^+$ 991 (6), 870 (2), 748 (2), 634 (2), 262 (1), 220 (3), 206 (16), 178 (9), 105 (100); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the National Science Council (NSC 82-0208-M-077-009) of Taiwan, Republic of China, awarded to Y.-H. Kuo. The authors thank the Cell Bank of Veterans General Hospital and Ms. Mei-Hsiang Huang, Taipei, for donations of the human tumor cell lines and suggestions for the biological assays, as well as Professor Muh-Tsuen Kao of Chinese Medicine, Taipei, for identification of the plant material.

LITERATURE CITED

1. Y.H. Kuo, C.H. Chen, L.M.Y. Kuo, M.L. King, T.S. Wu, S.T. Lu, I.S. Chen, D.R. McPhail, A.T. McPhail, and K.H. Lee, *Heterocycles*, **29**, 1465 (1989).
2. Y.H. Kuo, C.H. Chen, L.M.Y. Kuo, M.L. King, T.S. Wu, M. Haruna, and K.H. Lee, *J. Nat. Prod.*, **53**, 422 (1990).
3. Y.H. Kuo, C.H. Chen, M.L. King, T.S. Wu, and K.H. Lee, *Phytochemistry*, **35**, 803 (1994).
4. Y.H. Kuo, M.L. King, C.F. Chen, H.Y. Chen, C.H. Chen, and K.H. Lee, *J. Nat. Prod.*, **57**, 263 (1994).
5. Y.Q. Tu, *Phytochemistry*, **31**, 2155 (1992).
6. R. Bruning and H. Wagner, *Phytochemistry*, **17**, 1821 (1978).
7. N. Wakabayashi, W.J. Wu, R.M. Waters, R.E. Redfern, J.G.D. Mills, A.B. DeMilo, W.R. Lusby, and D. Andrzejewski, *J. Nat. Prod.*, **51**, 573 (1988).
8. T. Yoshihisa, A. Fumie, T. Shouji, N. Kimiko, and T. Toshiaki, *Phytochemistry*, **31**, 2155 (1992).
9. H.C. Barrett and G. Buchi, *J. Am. Chem. Soc.*, **89**, 5665 (1967).
10. A. Asselin, M. Mongrain, and P. Deslongchamps, *Can. J. Chem.*, **46**, 2817 (1968).

Received 16 February 1995