SESQUITERPENE PYRIDINE ALKALOIDS FROM MAYTENUS EBENIFOLIA

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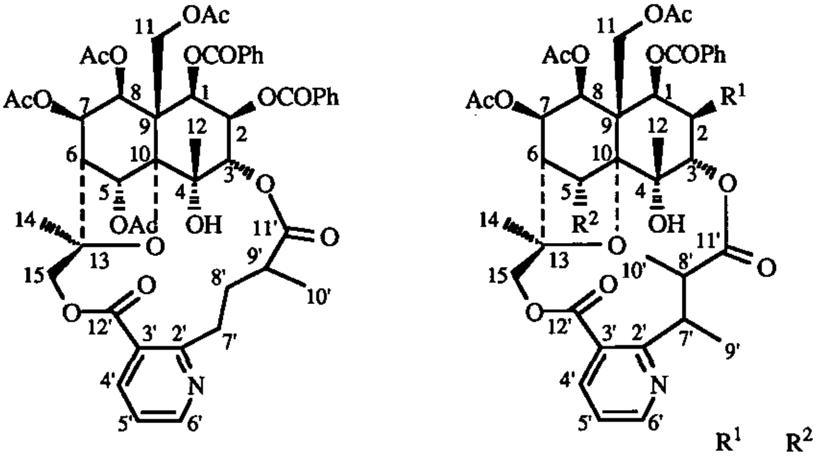
Abstract - Three new sesquiterpene pyridine alkaloids, ebenifolines W-I (**1**), E-I (**2**), and E-II (**3**) were isolated from the stem barks of Maytenus ebenifolia Reiss. (Celastraceae). Their structures were elucidated by spectroscopic methods.

In the course of our continuing research among Amazonian medicinal plants,[1,2] we had a interest in the genus Maytenus, because the plants classified into this genus had been used for various purposes as folk medicine, such as antitumor, antirheumatism and anti-inflammatory, by several tribes in Amazonian basin.[3,4]

We report herein the isolation and structural characterization of three new sesquiterpene pyridine alkaloids from the stem barks of Maytenus ebenifolia Reiss. (Celastraceae), which was used against rheumatism in Amazonian Peru, named as "chuchuhuasi".[3,4]



Ebenifoline W-I (**1**)

Ebenifoline E-I (**2**) : OH OAc

Ebenifoline E-II (**3**) : OAc OCOPh

Figure 1. The structures of ebenifolines.

Fractionation of CH_2Cl_2 soluble phase of the MeOH extract led us to the isolation of three new sesquiterpene pyridine alkaloids, ebenifolines W-I (**1**) (wilfordate type[5-7]), E-I (**2**) and E-II (**3**) (evoninate type[8-10]). A combination of ^1H-^1H COSY, ^{13}C-^1H COSY, COLOC, HMQC and HMBC[11] spectra enabled us to perform complete assignment of the ^1H- and ^{13}C-signals of **1**, **2** and **3**, as shown in Tables 1 and 2, respectively.

Table 1. ^{1}H Nmr chemical shifts (ppm) for ebenifolines W-I, E-I and E-II.

Proton	ebenifoline W-I	proton	ebenifoine E-I	ebenifoline E-II
1-H	6.13 (d, 3.9)	1-H	5.76 (d, 3.9)	5.94 (d, 3.9)
2-H	5.52 (dd, 2.4, 3.9)	2-H	4.13 (dd, 2.6, 3.9)	5.41 (dd, 2.5, 3.9)
3-H	5.16 (d, 2.4)	3-H	4.83 (d, 2.6)	4.83 (d, 2.5)
4-OH	4.99 (s)	4-OH	4.47 (br-s)	4.78 (s)
5-H	6.96 (s)	5-H	7.06 (s)	7.22 (s)
6-H	2.40 (d, 3.8)	6-H	2.35 (d, 4.0)	2.55 (d, 4.0)
7-H	5.57 (dd, 3.8, 5.8)	7-H	5.53 (dd, 4.0, 5.8)	5.58 (dd, 4.0, 5.7)
8-H	5.48 (d, 5.8)	8-H	5.41 (d, 5.8)	5.48 (d, 5.7)
11-Ha	4.61 (d, 13.3)	11-Ha	4.81 (d, 13.8)	4.76 (d, 13.4)
11-Hb	5.69 (d, 13.3)	11-Hb	5.47 (d, 13.8)	5.41 (d, 13.4)
12-CH$_3$	1.70 (s)	12-CH$_3$	1.63 (d, 1.2)	1.61 (s)
14-CH$_3$	1.74 (s)	14-CH$_3$	1.71 (s)	1.75 (s)
15-Ha	3.83 (d, 11.9)	15-Ha	3.69 (d, 11.7)	3.64 (d, 11.5)
15-Hb	5.77 (d, 11.9)	15-Hb	5.98 (d, 11.7)	6.05 (d, 11.5)
4'-H	8.34 (dd, 1.6, 8.0)	4'-H	8.06 (dd, 1.8, 7.8)	8.07 (dd, 1.7, 7.8)
5'-H	7.30 (dd, 4.7, 8.0)	5'-H	7.26 (dd, 4.9, 7.8)	7.27 (dd, 4.8, 7.8)
6'-H	8.77 (dd, 1.6, 4.7)	6'-H	8.69 (dd, 1.8, 4.9)	8.71 (dd, 1.7, 4.8)
7'-Ha	2.99 (ddd, 4.5, 6.2, 13.6)	7'-H	4.67 (q, 7.0)	4.74 (q, 6.9)
7'-Hb	3.97 (ddd, 6.3, 9.5, 13.6)	----	----	----
8'-Ha	2.04 (m)	8'-H	2.58 (q, 7.1)	2.64 (q, 7.1)
8'-Hb	2.32 (m)	----	----	----
9'-H	2.47 (m)	9'-CH$_3$	1.38 (d, 7.0)	1.46 (d, 6.9)
10'-CH$_3$	1.26 (d, 7.0)	10'-CH$_3$	1.17 (d, 7.1)	1.24 (d, 7.1)
1-OBz(o)	7.78 (d, 7.7)	1-OBz(o)	7.95 (dd, 1.3, 8.5)	7.84 (d, 7.1)
OBz(m)	7.32 (t-like, 7.8)	OBz(m)	7.42 (t-like, 7.8)	7.40 (t-like, 7.7)
OBz(p)	7.49 (t-like, 7.4)	OBz(p)	7.56 (dt-like, 1.3, 7.5)	7.54 (t-like, 7.4)
2-OAc	----	2-OAc	----	2.17 (s)
OBz(o)	8.09 (d, 7.7)	OBz(o)	----	----
OBz(m)	7.51 (t-like, 7.8)	OBz(m)	----	----
OBz(p)	7.63 (t-like)	OBz(p)	----	----
5-OAc	2.19 (s)	5-OAc	2.21 (s)	----
OBz(o)	----	OBz(o)	----	8.34 (d, 7.3)
OBz(m)	----	OBz(m)	----	7.50 (t-like, 7.7)
OBz(p)	----	OBz(p)	----	7.60 (t-like, 7.4)
7-OAc	2.14 (s)	7-OAc	2.12 (s)	2.21 (s)
8-OAc	1.36 (s)	8-OAc	1.40 (s)	1.43 (s)
11-OAc	2.21 (s)	11-OAc	2.37 (s)	2.37 (s)

Measurements were performed in CDCl$_3$ at 400 MHz.
Multiplicity and coupling constants (J/Hz) were in parenthesis.

Ebenifoline W-I (**1**)[12] was obtained as an amorphous solid, and its molecular formula $C_{48}H_{51}NO_{18}$ (m/z 929.3124) was determined by means of HR ms spectrum. The ir spectrum showed the presence of a hydroxyl group (3444 cm^{-1}) ascribable to an intramolecular hydrogen bond and several ester carbonyl groups (1750-1725 cm^{-1}). The ^{1}H-nmr spectrum (400 MHz, CDCl$_3$) showed the presence of two tertiary methyl groups (δ 1.70, H-12; δ 1.74, H-14), four acetyl methyl groups (δ 1.36, AcO-8; δ 2.14, AcO-7; δ 2.19, AcO-5; δ 2.21, AcO-11), two methylene groups (δ 4.61 and 5.69, H-11; δ 3.83 and 5.77, H-15), and seven methine protons (δ 6.13, H-1; δ 5.52, H-2; δ 5.16, H-3; δ 6.96, H-5; δ 2.40, H-6; δ 5.57, H-7; δ 5.48, H-8), suggesting a dihydro-β-agarofuran-type sesquiterpene similar to euonyminol.[8-10] In addition, the presence of one secondary methyl group (δ 1.26, H-10') coupled with one methine proton (δ 2.47, H-9'), two methylene groups (δ 2.99 and 3.97, H-7'; δ 2.04 and 2.32, H-8') coupled each other and a 2,3-disubstituted pyridine (δ 8.34, H-4'; δ7.30, H-5'; δ 8.77, H-6') were observed. This indicated the presence of a wilfoldate macrocyclic diester linkage[5-7] at C-3 and C-15 as a diabasic acid moiety. Moreover, the signals due to two benzoyl groups (δ 7.78, 7.32 and 7.49; δ 8.09, 7.51 and 7.63) were observed. In the COLOC spectrum, long range couplings of the carbonyl carbon signal at δ 165.02 with the C-1 methine proton signal at δ 6.13 and the aromatic proton signal at δ 7.78 indicated the presence of one benzoyl group at C-1. This benzoyl group gave rise to an unusual diamagnetic effect against acetyl methyl equatorially oriented on C-8. Another benzoyl group must be located at C-2, because the same long range couplings of the carbonyl carbon at δ 164.85 with the C-2 methine proton at δ 5.52 and the aromatic proton at δ 8.09 were observed. The lower chemical shift of C-11 acetyl methyl was considered to be caused by the anisotropic effect of this benzoyl group. These spectroscopic data corroborated the structure of ebenifoline W-I (Figure 1).

Table 2. ^{13}C-Nmr chemical shifts (ppm) for **1**, **2** and **3**.

Carbon	**1**		**2**		**3**	
1	73.47	(d)	75.49	(d)	73.51	(d)
2	70.51	(d)	70.11	(d)	69.21	(d)
3	75.94	(d)	78.39	(d)	75.86	(d)
4	69.88	(s)	70.64	(s)	70.72	(s)
5	73.85	(d)	74.07	(d)	74.89	(d)
6	51.18	(d)	50.52	(d)	50.40	(d)
7	69.03	(d)	69.07	(d)	69.11	(d)
8	71.65	(d)	71.78	(d)	71.55	(d)
9	52.54	(s)	53.00	(s)	52.72	(s)
10	93.89	(s)	94.62	(s)	93.83	(s)
11	60.83	(t)	60.47	(t)	60.15	(t)
12	23.15	(q)	23.13	(q)	22.91	(q)
13	84.71	(s)	84.24	(s)	84.44	(s)
14	17.97	(q)	18.47	(q)	18.35	(q)
15	70.39	(t)	70.17	(t)	70.04	(t)
2'	164.09	(s)	165.21	(s)	165.23	(s)
3'	124.09	(s)	125.45	(s)	125.20	(s)
4'	138.78	(d)	137.72	(d)	137.66	(d)
5'	121.20	(d)	121.17	(d)	121.14	(d)
6'	153.21	(d)	151.49	(d)	151.51	(d)
7'	33.49	(t)	36.58	(d)	36.48	(d)
8'	33.35	(t)	45.08	(d)	45.05	(d)
9'	38.55	(d)	12.05	(q)	11.90	(d)
10'	18.82	(q)	9.48	(q)	9.72	(q)
11'	175.12	(s)	174.71	(s)	173.93	(s)
12'	166.94	(s)	168.57	(s)	168.61	(s)
1-OC=O	165.02	(s)	164.98	(s)	164.62	(s)
ipso	129.33	(s)	129.35	(s)	129.21	(s)
ortho	129.62	(d)	129.81	(d)	129.58	(d)
meta	128.42	(d)	128.67	(d)	128.52	(d)
para	133.38	(d)	133.68	(d)	133.48	(d)
2-OC=O	164.85	(s)	---		165.23	(s)
CH_3	---		---		20.88	(q)
ipso	128.85	(s)	---		---	
ortho	129.96	(d)	---		---	
meta	128.83	(d)	---		---	
para	133.79	(s)	---		---	
5-OC=O	169.93	(s)	169.99	(s)	165.79	(s)
CH_3	21.61	(q)	21.71	(q)	---	
ipso	---		---		129.52	(s)
ortho	---		---		130.34	(d)
meta	---		---		128.83	(d)
para	---		---		133.58	(d)
7-OC=O	170.02	(s)	170.11	(s)	170.06	(s)
CH_3	20.99	(q)	21.02	(q)	21.03	(q)
8-OC=O	168.96	(s)	169.12	(s)	168.90	(s)
CH_3	19.88	(q)	20.01	(q)	19.90	(q)
11-OC=O	170.47	(s)	170.28	(s)	170.26	(s)
CH_3	21.23	(q)	21.52	(q)	21.47	(q)

Measurements were peformed in $CDCl_3$ at 100 MHz.
Multiplicity was in parenthesis.

Ebenifoline E-I (**2**)[13] was an amorphous solid which had the molecular formula $C_{41}H_{47}NO_{17}$ (m/z 825.2800). In the ^{1}H-nmr, the presence of euonyminol skeleton[8-10] as a sesquiterpene moiety was observed as well as that of ebenifoline W-I. But, instead of the wilfordate diester,[5-7] an evoninate diester[8-10] as a diabasic acid moiety was suggested on the basis of the presence of two secondary methyl signals (δ 1.17, J=7.1 Hz, H-10'; δ 1.38, J=7.0 Hz, H-9') and aromatic proton signals of a 2,3-disubstituted pyridine. Both ^1H- and ^{13}C-nmr spectra of **2** had great similarities to those of mayteine,[14] euojaponines A and C,[15] possessing C-1 benzoyl group and C-8 acetyl methyl group influenced by its diamagnetic effect. Since ebenifoline E-I contained four acetyl groups and one benzoyl group as indicated by ms, ^1H- and ^{13}C-nmr spectral data, it seemed that ebenifoline E-I was an evoninate type alkaloid, *i.e.*, one of acetyl groups in mayteine[14] was replaced by a hydroxyl group which was confirmed by the ir spectrum (3611 cm^{-1}). The position of replacement was easily assigned as at C-2 by the following evidences; there were no long range couplings observed between the C-2 methine proton and any acethyl carbonyl carbon, and three of them had clear correlations with the other methine protons (H-5,7 and 8) in the COLOC spectrum. Furthermore, by the comparison of ^1H-nmr spectra between **2** and mayteine, H-2 methine proton of **2** was shifted upfield about 1.2 ppm than that of mayteine. This also supported the structure of ebenifoline E-I (Figure 1).

Ebenifoline E-II (**3**)[16] was an amorphous solid had the molecular formula, $C_{48}H_{51}NO_{18}$ (m/z 929.3086), like as ebenifoline W-I. The ^1H-nmr spectrum showed two secondary methyl signals (δ 1.24, J=7.1 Hz, H-10'; δ 1.46, J=7.0 Hz, H-9') which were characteristic of the evoninate diester moiety,[8-10] two sets of benzoyl, four acetyl methyl and two singlet methyl groups. Its sesquiterpene moiety was easily presumed as euonyminol type,[8-10] but the position of two benzoyl groups was seemed to be different from those of **1**. Compared of the ^1H-nmr data of sesquiterpene moiety of E-II with those of **1**, H-1, -2 and -3 methine protons of E-II were shifted upfield about 0.1-0.2 ppm, while H-5 and -6 methine protons were shifted downfield about 0.1-0.2 ppm. It suggested that E-II had a sesquiterpene moiety, whose C-1 or C-2 benzoyl group in **1** was replaced by C-5 acetyl group. This presumption was confirmed clearly by means of HMBC[11] spectrum, which showed cross peaks among each methine, aromatic proton and the corresponding benzoyl carbonyl carbon.

In order to determine the absolute configuration of **1**, the exciton chirality method[17] was applied for its 1,2-dibenzoate system. The cd spectrum exhibited a split cd curve, *i.e.*, positive first Cotton effect at 240 nm ($\Delta\varepsilon$=+22.6) and negative second Cotton effect at 223 nm ($\Delta\varepsilon$=-15.1). Thus the absolute configuration of **1** was determined as shown in Figure 1.

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13. an amorphous solid, mp 183-185°C (EtOH); cd λ max/EtOH nm($\Delta\varepsilon$) 246(+3.3), 227(-3.8); ir ν max/$CHCl_3$ cm^{-1} 3611, 3501, 1741(m); uv λ max/EtOH nm(log ε) 201(4.57), 229(4.40), 264(3.73).
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