SESQUITERPENE PYRIDINE ALKALOIDS FROM MAYTENUS CHUCHUHUASCA

Osamu Shirota, Akiko Otsuka, Hiroshi Morita, Koichi Takeya, and Hideji Itokawa*

Department of Pharmacognosy, Tokyo College of Pharmacy, Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan

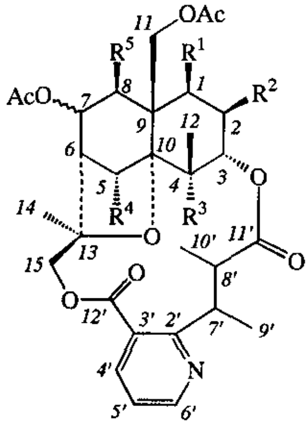
Abstract - Structural elucidations of seven novel sesquiterpene pyridine alkaloids, chuchuhuanines E-I (**1**), E-II (**2**), E-III (**3**), E-IV (**4**), E-V (**5**), W-I (**6**) and 4-deoxyeuonymine (**7**), which were isolated along with seven known alkaloids from root barks of Maytenus chuchuhuasca Raymond-Hamet et Colas (Celastraceae), are presented. Their structures with a novel dihydro-β-agarofuran type sesquiterpene core, linking several ester moieties and including a fifteen or a sixteen membered macrocyclic diester ring system, were elucidated by mainly spectroscopic methods.

During our studies on biologically active compounds in South American medicinal plants,[1] recently we have focused on searching for the chemical constituents of the genus Maytenus in plants of family Celastraceae.[2-7] This species is widely used as folk medicines in South America,[8,9] and many characteristic bioactive compounds, such as the maytansinoids[10] with antitumor activity, quinoid triterpenes[2,11] with cytotoxic activity, sesquiterpene polyesters[12] with anti-tumor-promoting activity, and sesquiterpene pyridine alkaloids[13] with insect antifeedant or insecticidial activity, so far have been isolated and reported. Besides, the sesquiterpene pyridine alkaloids isolated from Tripterygium species that possessed the immunosuppressive activity[14] were also reported.

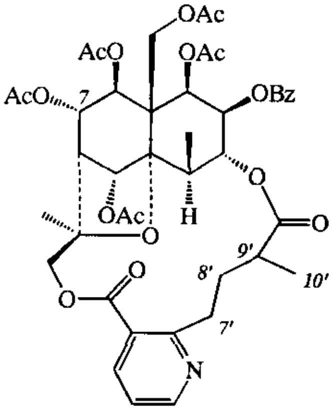
In our previous studies concerned with sesquiterpene pyridine alkaloids, we have reported isolations from Paraguayan medicinal plant "cangorosa" (M. ilicifolia)[7] and Peruvian medicinal plant "chuchuhuasi" (M. ebenifolia),[4,5] structural determination and conformational analyses of them.

In this paper, we report the chemical study of the another material, Brazilian medicinal plant "xuxuá" (Maytenus chuchuhuasca Raymond-Hamet et Colas.),[9,15] which are used for the treatment of skin cancer. From this plant, fourteen sesquiterpene pyridine alkaloids (thirteen evoninate types[16-18] and one wilfordate type[19-21]) including seven novel ones, named as chuchuhuanines E-I (**1**), E-II (**2**), E-III (**3**), E-IV (**4**), E-V (**5**), W-I (**6**) and 4-deoxyeuonymine (**7**), and seven known ones, ebenifolines E-I (**8**), E-II (**9**), E-III (**10**), E-VI (**11**), E-V (**12**), mayteine (**13**) and euonymine (**14**),[4,5] were isolated. Their structures were elucidated by chemical and spectroscopic methods.

A methylene chloride soluble portion of a methanolic extract of Maytenus chuchuhuasca (5 kg) was subjected to a silica gel column chromatography. The fractions obtained were further separated by silica gel and/or reverse-phase silica gel medium pressure liquid chromatography (mplc), following high performance liquid chromatography (hplc) to give seven new sesquiterpene pyridine alkaloids, chuchuhuanines E-I (**1**: 0.30%), E-II (**2**: 0.052%), E-III (**3** 0.0009%), E-IV (**4**: 0.0039%), E-V (**5**: 0.0011%), W-I (**6**: 0.0027%), and 4-deoxyeuonymine (**7**: 0.0013%), and also seven known alkaloids, ebenifolines E-I (**8**: 0.0064%), E-II (**9**: 0.0005%), E-III (**10**: 0.0053%), E-IV (**11**: 0.0018%), E-V (**12**: 0.0040%), mayteine (**13**: 0.030%) and euonymine (**14**: 0.026%).



	R1	R2	R3	R4	R5	C7
1 :	OAc	OAc	H	OAc	OAc	α
2 :	OBz	OAc	H	OAc	OAc	α
3 :	OAc	OBz	H	OAc	OAc	α
4 :	OAc	OAc	H	OH	OAc	α
5 :	OAc	OH	H	OAc	OAc	α
7 :	OAc	OAc	H	OAc	OAc	β
8 :	OBz	OH	OH	OAc	OAc	β
9 :	OBz	OAc	OH	OBz	OAc	β
10 :	OBz	OAc	OH	OAc	OBz	β
11 :	OBz	OAc	H	OAc	OAc	β
12 :	OBz	OAc	OH	OH	OBz	β
13 :	OBz	OAc	OH	OAc	OAc	β
14 :	OAc	OAc	OH	OAc	OAc	β



6

Figure 1. Structures of sesquiterpene pyridine alkaloids isolated from M. chuchuhuasca.

Chuchuhuanine E-I (**1**) was obtained as colorless plates with a molecular formula, $C_{38}H_{47}NO_{17}$. It contained six acetyl groups [δ_H : 1.82, 1.93, 1.96, 2.09, 2.18, 2.25 (each s)], one secondary methyl group [δ_H : 1.20 (d, J = 7.9 Hz)], one tertiary methyl group [δ_H : 1.54 (s)], two sets of methylene protons [δ_H : 3.87, 5.21 (each d, J = 11.1 Hz); 4.63, 4.66 (each d, J = 12.9 Hz)], eight methine protons [δ_H : 2.49 (d, J = 3.0 Hz), 2.70 (q, J = 7.9 Hz), 4.75 (br s), 5.33 (br s), 5.54 (dd, J = 3.0, 9.6 Hz), 5.60 (d, J = 9.6 Hz), 5.67 (d, J = 3.5 Hz), 6.32 (s)], as a sesquiterpene core based on a dihydro-β-agarofuran. It also contained one 2, 3-disubstituted pyridine unit [δ_H :7.20 (dd, J = 4.7, 7.8 Hz), 7.95 (dd, J = 1.5, 7.8 Hz), 8.63 (dd, J = 1.5, 4.7 Hz)], two secondary methyl groups [δ_H : 1.24 (d, J = 7.4 Hz), 1.38 (d, J = 6.9 Hz)] and two methine protons [δ_H : 2.75 (q, J = 7.4 Hz), 4.15 (q, J = 6.9 Hz)] which are coupling each other, corresponding to an evoninic acid.[16-18] These data indicated that this was a sesquiterpene pyridine alkaloid derived from polyester sesquiterpenes, which are characteristically detected in Celastraceae plants. It included one macrocyclic structure formed by two ester linkages between one sesquiterpene molecule and one evoninic acid, at positions 3 and 15. Besides, six acetyl groups were presumably linked at C-1, C-2, C-5, C-7, C-8 and C-11 on the sesquiterpene core. The most of sesquiterpene pyridine alkaloids possess an euonyminol[16-18] as a dihydro-β-agarofuran sesquiterpene core, the orientations of ester groups are 1βeq, 2βax, 5αeq, 7βax and 8βeq, and contain a hydroxyl group at C-4. This compound, however, differed from euonyminol skeleton with respected to the ^1H coupling constants among H-6, H-7 and H-8 methine protons, and lacking of 4-hydroxyl group. In the euonyminol skeleton, while their couplings are generally $J_{6,7}$ = 3.8 – 4.0 Hz and $J_{7,8}$ = 5.6 – 5.9 Hz, this chuchuhuanine E-I (**1**) showed $J_{6,7}$ = 3.0 Hz and $J_{7,8}$ = 9.6 Hz for the coupling constants among H-6, H-7 and H-8 (Figure 2). Furthermore, H-7 methine proton showed a NOE correlation with H-5 methine proton, and H-8 showed it with H-14 methyl proton. These data indicated that the configuration of the ester group at C-7 was α equatorial orientation. Therefore, the structure of chuchuhuanine E-I was determined as **1**.

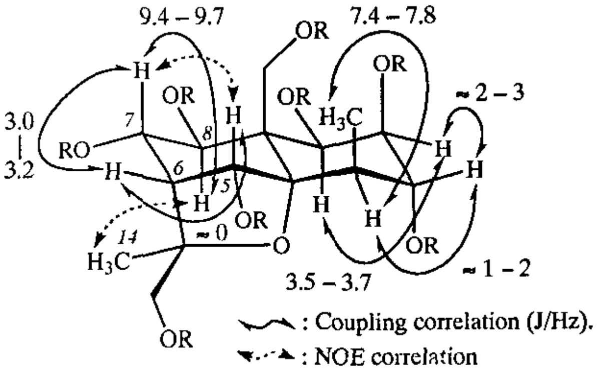
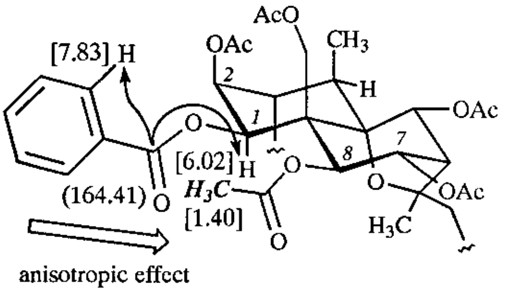


Figure 2. Configuration of a sesuqiterpene core on chuchuhuanines E-I (**1**) – E-V (**5**), and W-I (**6**).

Chuchuhuanine E-II (**2**) was an amorphous solid, with a molecular formula $C_{43}H_{49}NO_{17}$. The nmr spectra suggested that it was an evoninate type sesquiterpene pyridine alkaloid having five acetyl groups [δ_H : 1.40, 1.92, 2.12; 2.22, 2.30 (each s)] and one benzoyl group [δ_H : 7.40 (2H, t-like, J = 7.9 Hz; *meta*), 7.51 (1H, t-like, J = 7.4 Hz; *para*), 7.83 (2H, dd, J = 1.4, 7.8 Hz; *ortho*)]. Its sesquiterpene core was also 7αeq and 4-deoxy type, which was identical with that of chuchuhuanine E-I (**1**). The benzoyl group gave an anisotropic effect to one acetyl group (δ_H : 1.40), and was determined to be at C-1 as shown in Figure 3 by COLOC spectrum. That is, the carbonyl carbon at δ_C 164.41 gave cross peaks with the methine proton (δ_H : 6.02) of position 1 and the ortho protons (δ_H : 7.83) of benzoyl group. These data showed that the structure of chuchuhuanine E-II (**2**) was as shown in Figure 1.



↷ : $^3J_{C,H}$ correlation

^1H and ^{13}C-nmr assignment in parenthesis.
[δ_H, ppm, in $CDCl_3$], (δ_C, ppm, in $CDCl_3$)

Figure 3. Partial structure of chuchuhuanine E-II (**2**).

Chuchuhuanine E-III (**3**), an amorphous solid with a molecular formula $C_{43}H_{49}NO_{17}$, was an evoninate type sesquiterpene pyridine alkaloid contained five acetyl groups and one benzoyl group as ester groups on the chuchuhuaninol, similar to **2**. However, the benzoyl group did not give any anisotropic effects to acetyl groups, so this phenomenon suggested that this benzoyl group positioned at neither C-1 nor C-8. In the COLOC spectrum, the cross peaks between the benzoyl carbonyl carbon at δ_C 165.06, and H-2 methine proton (δ_H 5.65) were observed. Therefore, the structure of chuchuhuanine E-III was determined as **3**.

Chuchuhuanine E-IV (**4**), an amorphous solid, $C_{36}H_{45}NO_{16}$, was an evoninate type sesquiterpene pyridine alkaloid, and its sesquiterpene core also contained five acetyl groups. Further oxygenated functional group was assumed to be a hydroxyl group. The position of this hydroxyl group was confirmed at C-5, by the reason that

Table 1. ^{1}H-Nmr chemical shifts (ppm) for chuchuhuanines E-I – V (**1** – **5**) and 4-deoxyeuonymine (**7**).

Proton	**1**	**2**	**3**	**4**	**5**	**7**
1-H	5.67 (d, 3.5)	6.02 (d, 3.7)	5.84 (d, 3.7)	5.68 (d, 3.6)	5.50 (d, 3.5)	5.67 (d, 4.0)
2-H	5.33 (br s)	5.48 (br s)	5.65 (br s)	5.34 (br s)	4.09 (br s)	5.30 (br s)
3-H	4.75 (br s)	4.85 (br s)	4.92 (br s)	4.72 (br s)	4.81 (br s)	4.77 (br s)
4-H	2.70 (q, 7.9)	2.80 (q, 8.0)	2.78 (q, 7.6)	2.75 (q, 7.8)	2.63 (q, 7.9)	2.68 (q, 7.9)
5-H	6.32 (s)	6.36 (s)	6.40 (s)	5.06 (d, 4.8)	6.30 (s)	6.72 (s)
6-H	2.49 (d, 3.0)	2.49 (d, 3.2)	2.54 (d, 3.2)	2.47 (d, 3.1)	2.47 (d, 3.0)	2.39 (d, 3.9)
7-H	5.54 (dd, 3.0, 9.6)	5.60 (dd, 3.2, 9.6)	5.59 (dd, 3.2, 9.6)	5.38 (dd, 3.1, 9.7)	5.51 (dd, 3.2, 9.4)	5.49 (dd, 3.9, 5.9)
8-H	5.60 (d, 9.6)	5.74 (d, 9.6)	5.70 (d, 9.6)	5.58 (d, 9.7)	5.58 (d, 9.4)	5.30 (d, 5.9)
11-Ha	4.63 (d, 12.9)	4.81 (d, 13.0)	4.59 (d, 12.9)	4.53 (d, 13.0)	4.75 (d, 14.3)	4.46 (d, 13.3)
11-Hb	4.66 (d, 12.9)	4.95 (d, 13.0)	5.05 (d, 12.9)	4.77 (d, 13.0)	4.79 (d, 14.3)	5.07 (d, 13.3)
12-CH$_3$	1.20 (d, 7.9)	1.28 (d, 8.0)	1.30 (d, 7.6)	1.37 (d, 7.3)	1.21 (d, 7.9)	1.17 (d, 7.9)
14-CH$_3$	1.54 (s)	1.59 (s)	1.59 (s)	1.48 (s)	1.52 (s)	1.48 (s)
15-Ha	3.87 (d, 11.1)	3.97 (d, 11.0)	3.93 (d, 11.1)	3.99 (d, 11.1)	3.75 (d, 11.0)	3.93 (d, 11.1)
15-Hb	5.21 (d, 11.1)	5.21 (d, 11.0)	5.25 (d, 11.1)	5.47 (d, 11.1)	5.34 (d, 11.0)	5.14 (d, 11.1)
4'-H	7.95 (dd, 1.5, 7.8)	8.00 (dd, 1.8, 7.8)	8.05 (dd, 1.7, 7.8)	7.93 (dd, 1.8, 7.8)	7.92 (dd, 1.8, 7.8)	7.94 (dd, 1.8, 7.8)
5'-H	7.20 (dd, 4.7, 7.8)	7.24 (dd, 4.8, 7.8)	7.22 (dd, 4.8, 7.8)	7.19 (dd, 4.8, 7.8)	7.18 (dd, 4.8, 7.8)	7.20 (dd, 4.8, 7.8)
6'-H	8.63 (dd, 1.5, 4.7)	8.68 (dd, 1.8, 4.8)	8.67 (dd, 1.7, 4.8)	8.62 (dd, 1.8, 4.8)	8.60 (dd, 1.8, 4.8)	8.63 (dd, 1.8, 4.8)
7'-H	4.15 (q, 6.9)	4.19 (q, 6.9)	4.19 (q, 6.9)	4.23 (dq, 1.0, 7.8)	4.18 (q, 7.0)	4.09 (q, 6.4)
8'-H	2.75 (q, 7.4)	2.84 (dq, 1.0, 7.4)	2.82 (q, 7.2)	2.84 (dq, 1.0, 7.4)	2.66 (dq, 1.0, 7.9)	2.76 (dq, 1.0, 7.4)
9'-CH$_3$	1.38 (d, 6.9)	1.44 (d, 6.9)	1.42 (d, 6.9)	1.37 (d, 7.3)	1.34 (d, 7.0)	1.38 (d, 7.0)
10'-CH$_3$	1.24 (d, 7.4)	1.31 (d, 7.4)	1.30 (d, 7.6)	1.27 (d, 7.4)	1.17 (d, 7.3)	1.24 (d, 7.3)
OH	----	----	----	3.55 (d, 4.8)	3.39 (br s)	----
OAc	1.82 (s) 1.93 (s) 1.96 (s) 2.09 (s) 2.18 (s) 2.25 (s)	1.40 (s) 1.92 (s) 2.12 (s) 2.22 (s) 2.30 (s) ----	1.82 (s) 1.94 (s) 1.99 (s) 2.21 (s) 2.30 (s) ----	1.83 (s) 1.90 (s) 1.94 (s) 2.09 (s) 2.14 (s) ----	1.88 (s) 1.93 (s) 1.95 (s) 2.16 (s) 2.22 (s) ----	1.84 (s) 1.95 (s) 2.10 (s) 2.13 (s) 2.17 (s) 2.22 (s)
OBz(o)	----	7.83 (dd, 1.4, 7.8)	7.99 (dd, 1.7, 7.8)	----	----	----
(m)	----	7.40 (t-like, 7.9)	7.49 (t-like, 7.9)	----	----	----
(p)	----	7.51 (t-like, 7.4)	7.60 (t-like, 7.4)	----	----	----

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

the H-5 methine proton (δ_H: 5.06, d, J = 4.8 Hz) was resonated at higher field than the corresponding proton of **1** and was shifted to lower field (Δ 1.4 ppm) by the addition of TAI (trichloroacetyl isocyanate) reagent.[22] These data matched with the structure of chuchuhuanine E-IV (**4**) shown in Figure 1.

Chuchuhuanine E-V (**5**), also an evoninate type sesquiterpene pyridine alkaloid, showed the same molecular formula, $C_{36}H_{45}NO_{16}$ as **4**, containing five acetyl groups and one hydroxyl group in the sesquiterpene core. The position of the hydroxyl group was determined to be at C-2 by the same argument as described above, which included the lower field shift of H-2 by the reaction with TAI reagent. In this manner, the structure of chuchuhuanine E-V (**5**) was decided.

Compound **7** was also an evoninate type sesquiterpene pyridine alkaloid, having a molecular formula, $C_{38}H_{47}NO_{17}$. In its molecule, six acetyl groups were appeared, analogous to those in chuchuhuanine E-I (**1**). It contained 4-deoxy type sesquiterpene skeleton, however, it was not 7αeq type skeleton but 4-deoxyeuonyminol with 7βax type skeleton. Therefore the structure of compound **7** was assigned to be 4-deoxyeuonymine.

Table 2. ^{1}H-Nmr chemical shifts (ppm) for chuchuhuanine W-I (**6**).

Proton			
1-H	5.84 (d, 3.7)	6'-H	8.68 (dd, 1.8, 4.7)
2-H	5.53 (br s)	7'-Ha	3.00 (ddd, 5.1, 6.7, 13.7)
3-H	4.96 (br s)	7'-Hb	3.70 (ddd, 6.7, 9.4, 13.7)
4-H	2.76 (q, 7.9)	8'-Ha	2.0 (m)
5-H	6.34 (s)	8'-Hb	2.1 (m)
6-H	2.52 (d, 3.0)	9'-H	2.35 (m)
7-H	5.64 (dd, 3.3, 9.6)	10'-CH_3	1.15 (d, 7.1)
8-H	5.71 (d, 9.6)	OAc	1.82 (s)
11-Ha	4.60 (d, 12.9)		1.93 (s)
11-Hb	5.00 (d, 12.9)		1.99 (s)
12-CH_3	1.30 (d, 7.9)		2.17 (s)
14-CH_3	1.66 (s)		2.28 (s)
15-Ha	3.61 (d, 11.4)	OBz(o)	8.03 (dd, 1.3, 8.5)
15-Hb	5.48 (d, 11.4)	(m)	7.47 (t-like, 7.7)
4'-H	8.19 (dd, 1.8, 7.9)	(p)	7.58 (tt-like, 1.0, 8.2)
5'-H	7.21 (dd, 4.7, 7.9)		

Measurements performed in $CDCl_3$ at 400 MHz.
Multiplicity and coupling constants (J/Hz) given in parenthesis.

Table 3. ^{13}C-Nmr chemical shifts (ppm) for chuchuhuanines E-I – V (**1** – **5**), W-I (**6**), and 4-deoxycuonymine (**7**).

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	72.87 (d)*	73.26 (d)*	72.99 (d)	73.35 (d)*	75.20 (d)*	72.79 (d)	73.88 (d)*
2	69.25 (d)	69.74 (d)	69.98 (d)	70.65 (d)	69.41 (d)	70.34 (d)	69.08 (d)#
3	73.15 (d)*	73.34 (d)*	73.42 (d)	73.69 (d)*	73.56 (d)	73.84 (d)	74.20 (d)*
4	35.83 (d)	35.97 (d)	35.79 (d)	35.84 (d)	35.83 (s)	36.80 (d)	35.89 (d)
5	75.00 (d)	75.30 (d)	75.09 (d)	74.52 (d)#	75.60 (d)*	75.08 (d)	74.05 (d)*
6	49.11 (d)	49.49 (d)	49.29 (d)	51.13 (d)	49.12 (d)	49.40 (d)	50.29 (d)
7	73.27 (d)*	73.41 (d)*	73.65 (d)	73.85 (d)*	74.63 (d)	73.40 (d)	69.50 (d)#
8	74.21 (d)	74.54 (d)	74.28 (d)	74.45 (d)#	75.30 (d)*	74.16 (d)	70.92 (d)#
9	50.04 (s)	50.65 (s)	49.90 (s)	50.03 (s)	50.43 (s)	49.75 (s)	50.73 (s)
10	90.71 (s)	90.95 (s)	90.92 (s)	91.98 (s)	91.26 (s)	90.91 (s)	90.57 (s)
11	59.95 (t)	60.15 (t)	60.87 (t)	60.26 (t)	60.65 (t)	60.90 (t)	59.84 (t)
12	14.65 (q)	14.92 (q)	15.00 (q)	15.49 (q)	14.86 (q)	15.61 (q)	14.03 (q)
13	83.61 (s)	83.99 (s)	83.84 (s)	83.41 (s)	83.36 (s)	84.13 (s)	82.23 (s)
14	18.96 (q)	19.12 (q)	19.19 (q)	19.13 (q)	19.16 (q)	19.08 (q)	18.13 (q)
15	69.35 (t)	69.68 (t)	69.56 (t)	69.65 (t)	69.41 (t)	69.41 (t)	69.58 (t)
2'	163.66 (s)	163.88 (s)	163.91 (s)	163.38 (s)	163.76 (s)	162.73 (s)	163.56 (s)
3'	126.38 (s)	126.56 (s)	126.53 (s)	126.91 (s)	126.62 (s)	125.62 (s)	126.58 (s)
4'	137.64 (d)	137.95 (d)	137.86 (d)	137.77 (d)	137.66 (d)	138.46 (d)	137.73 (d)
5'	120.82 (d)	121.00 (d)	120.96 (d)	120.94 (d)	120.91 (d)	120.86 (d)	120.99 (d)
6'	150.81 (d)	151.08 (d)	151.03 (d)	150.80 (d)	150.80 (d)	152.39 (d)	150.96 (d)
7'	36.93 (d)	37.30 (d)	37.18 (d)	37.33 (d)	36.93 (d)	32.88 (t)	37.19 (d)
8'	43.51 (d)	43.58 (d)	43.68 (d)	43.18 (d)	43.75 (d)	33.11 (t)	43.53 (d)
9'	12.33 (q)	12.59 (q)	12.55 (q)	12.77 (d)	12.35 (q)	37.64 (d)	12.51 (q)
10'	10.10 (q)	10.45 (q)	10.39 (q)	10.42 (q)	9.79 (q)	18.77 (q)	10.28 (q)
11'	175.17 (s)	175.55 (s)	175.41 (s)	175.68 (s)	175.68 (s)	175.69 (s)	175.40 (s)
12'	167.42 (s)	167.76 (s)	167.69 (s)	168.16 (s)	167.46 (s)	166.13 (s)	167.72 (s)
AcO(CO)	168.56 (s)	168.48 (s)	168.98 (s)	168.89 (s)	169.37 (s)	169.15 (s)	168.82 (s)
	168.76 (s)	169.37 (s)	169.34 (s)	169.16 (s)	169.54 (s)	169.35 (s)	168.87 (s)
	169.12 (s)	169.48 (s)	169.61 (s)	169.16 (s)	169.75 (s)	169.66 (s)	169.01 (s)
	169.39 (s)	169.71 (s)	169.71 (s)	169.80 (s)	169.81 (s)	169.69 (s)	169.47 (s)
	169.48 (s)	170.10 (s)	170.10 (s)	170.10 (s)	170.09 (s)	170.06 (s)	169.96 (s)
	169.70 (s)	---	---	---	---	---	170.04 (s)
(CH$_3$)	20.31 (q)	20.11 (q)	20.54 (q)	20.55 (q)	20.63 (q)	20.54 (q)	20.36 (q)
	20.45 (q)	20.72 (q)	20.70 (q)	20.55 (q)	20.71 (q)	20.69 (q)	20.38 (q)
	20.56 (q)	20.82 (q)	20.80 (q)	20.78 (q)	20.78 (q)	20.75 (q)	20.91 (q)
	20.70 (q)	21.20 (q)	21.10 (q)	20.90 (q)	21.12 (q)	21.04 (q)	20.91 (q)
	20.92 (q)	21.30 (q)	21.26 (q)	21.22 (q)	21.16 (q)	21.18 (q)	21.23 (q)
	21.04 (q)	---	---	---	---	---	21.34 (q)
BzO(CO)	---	164.41 (s)	165.06 (s)	---	---	165.73 (s)	---
(ipso)	---	129.69 (s)	128.92 (s)	---	---	129.80 (s)	---
(ortho)	---	129.37 (d)	129.87 (d)	---	---	128.76 (d)	---
(meta)	---	128.46 (d)	128.76 (d)	---	---	128.70 (d)	---
(para)	---	133.20 (d)	133.65 (d)	---	---	133.63 (d)	---

Measurements performed in CDCl$_3$ at 100 MHz.
Multiplicity given in parenthesis.
*, # : These sets of values may be changeable in each column.

Chuchuhuanine W-I (**6**) was a sesquiterpene pyridine alkaloid that had a molecular formula $C_{47}H_{50}NO_{18}$. Its nicotinate derivative unit in the macrocycle was not evoninic acid but wilfordic acid,[19-21] which was representative with one secondary methyl group [δ_H : 1.15 (d, J = 7.1 Hz)], one methine proton [δ_H · 2.35 (m)] and two sets of methylenes [δ_H : 2.0, 2.1 (each m); 3.00 (ddd, J = 5.1, 6.7, 13.7 Hz), 3.70 (ddd, 6.7, 9.4, 13.7 Hz)] coupled each other. It contained five acetyl groups and one benzoyl group in the sesquiterpene core with 7αeq orientation. The position of the benzoyl group was determined by long range correlation data among the corresponding carbon and proton obtained from a COLOC spectrum, to be at C-2. These data showed that the structure of chuchuhuanine W-I (**6**) was shown in Figure 1.

Structures of ebenifolines E-I (**8**), E-II (**9**), E-III (**10**), E-IV (**11**), E-V (**12**), mayteine (**13**) and euonymine (**14**) were identified by the direct comparison with the spectroscopic data of the authentic samples.[4,5]

EXPERIMENTAL

General Details. - Mp's were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 spectrometer and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2g^{-1}. Mass, uv, ir, and cd spectra were taken with a Hitachi M-80 spectrometer, a Hitachi 557 spectrophotometer, a JASCO A-302 spectrophotometer and JASCO J-700 spectropolarimeter, respectively. Medium-pressure liquid chromatography (mplc) was performed with a CIG column system (22 mm i.d. × 300 mm, Kusano Scientific Co., Tokyo) packed with 10 μm Si gel or 20 μm ODS. Hplc was performed with an Inertsil PREP-ODS column (20 mm i.d. × 250 mm, GL Science Inc.) packed with 10 μm ODS. Tlc was conducted on precoated Kieselgel 60 F_{254} (Art. 5715; Merck) and the spots were detected by heating after spraying with 10% H_2SO_4. ^1H and ^{13}C nmr spectra were recorded on Bruker spectrometers (AM 400 and AM 500) at 303 K and processed on a Bruker data station with an Aspect 3000 computer. The nmr coupling constants (J) are given in Hz.

Materials. - Dark reddish to brown stem barks of Maytenus chuchuhuasca Raymond-Hamet et Colas (5 kg), commonly known as "xuxuá", were purchased at São Paulo, Brazil in 1992. The botanical identification was made by Dr. William Antonio Rodrigues (Instituto Nacional de Pesquisas da Amazonia). A voucher specimen has been deposited in the herbarium of the Tokyo College of Pharmacy.

Extraction and isolation of **1** - **14**. - The barks (5 kg) of M. chuchuhuasca were crushed and extracted with a hot MeOH (18 l x 3, each 6 hours) to give a MeOH extract (1.5 kg), which was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble fraction (155 g) was subjected to silica gel cc using an CH_2Cl_2/ ethyl acetate gradient system (1:0 - 0:1) to give twelve fractions. Fractions VII and VIII were further subjected to silica gel cc and/or ODS mplc to give **1** (15 g), **2** (2.6 g), **3** (45 mg), **4** (193 mg), **5** (54 mg), **6** (136 mg), **7** (67 mg), **8** (320 mg), **9** (26 mg), **10** (266 mg), **11** (89 mg), **12** (200 mg), **13** (1.5 g) and **14** (1.3 g). These compounds were further purified by ODS hplc with MeOH / H_2O or acetonitrile / H_2O solvent systems.

Chuchuhuanine E-I (**1**) - Colorless plates; mp, 247 – 250°C, $[\alpha]_D$ -23.3° (c 0.77, $CHCl_3$); cd λ max (MeOH) nm (Δε), 275.9 (+0.8), 257 (-0.1), 242 (+3.2), 229 (+4.0); ms m/z (%), 789 (M^+, 29, calcd, $C_{38}H_{47}NO_{17}$: 789.2844; found : 789.2834), 730 (31), 686 (7), 259 (13), 229 (10), 206 (38), 178 (24), 134 (28), 107 (100); ir ν max ($CHCl_3$) cm^{-1}, 1768 (br s), 1585 (s), 1568(s); uv λ max (MeOH) nm (log ε), 202 (4.02), 212 (3.95), 261 (3.64); ^1H-nmr ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

Chuchuhuanine E-II (**2**) - Colorless amorphous solid; mp, 158 – 160°C; $[\alpha]_D$ -8.5° (c 0.46, $CHCl_3$); cd λ max (MeOH) nm (Δε), 245 (+7.8), 222 (-6.7); ms m/z (%), 851 (M^+, 12, calcd, $C_{43}H_{49}NO_{17}$: 851.3001; found : 851.3014), 792 (10), 381 (7), 259 (10), 218 (21), 206 (27), 178 (29), 134 (31), 105 (100); ir ν max ($CHCl_3$) cm^{-1}, 1762 (br s), 1603 (s), 1585 (s), 1568 (s); uv λ max (MeOH) nm (log ε), 202 (4.25), 228 (4.28), 261 (3.63); ^1H-nmr ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

Chuchuhuanine E-III (**3**) - Colorless amorphous solid; mp, 156 – 160°C, $[\alpha]_D$ -412.5° (c 0.04, $CHCl_3$); cd λ max (MeOH) nm (Δε), 274 (+0.8), 259 (-0.6), 229 (+8.8), 211 (-0.7); ms m/z (%), 851 (M^+, 6, calcd, $C_{43}H_{49}NO_{17}$: 851.3001; found : 851.3022), 792 (7), 367 (3), 269 (6), 219 (9), 206 (12), 178 (13), 169 (21), 105 (100); ir ν max ($CHCl_3$) cm^{-1}, 1751 (br s), 1602 (s); uv λ max (MeOH) nm (log ε), 203 (4.18), 229 (4.31), 263 (3.65); ^1H-nmr ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

Chuchuhuanine E-IV (**4**) - Colorless amorphous solid; mp, 162 – 166°C; $[\alpha]_D$ –9.3° (c 0.51, $CHCl_3$); cd λ max (MeOH) nm (Δε), 268 (+2.3), 232 (+6.6), 211 (-3.1); ms m/z (%), 747 (M^+, 77, calcd, $C_{36}H_{45}NO_{16}$: 747.2738; found : 747.2762), 674 (55), 259 (21), 206 (100), 161 (82); ir ν max ($CHCl_3$) cm^{-1}, 3614 (br s), 1763 (br s), 1586 (s), 1568(s); uv λ max (MeOH) nm (log ε), 202 (3.91), 210 (3.86), 261 (3.52); ^1H-nmr ($CDCl_3$, 400

MHz), listed in Table 1; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

Chuchuhuanine E-V (5) - Colorless amorphous solid; mp, 154 – 160°C, $[\alpha]_D$ -18.3° (c 0.45, $CHCl_3$); cd λ max (MeOH) nm ($\Delta\varepsilon$), 274 (+0.6), 241 (+2.2), 210 (-2.7); ms m/z (%), 747 (M^+, 26, calcd, $C_{36}H_{45}NO_{16}$: 747.2738 ; found : 747.2721), 688 (24), 614 (16), 269 (17), 219 (30), 206 (49), 178 (38), 169 (44), 107 (100); ir ν max ($CHCl_3$) cm^{-1}, 3615 (br s), 1762 (br s), 1585 (s), 1568 (s); uv λ max (MeOH) nm (log ε), 202 (3.87), 213 (3.76), 264 (3.43); ^{1}H-nmr ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

Chuchuhuanine W-I (6) - Colorless amorphous solid; mp, 149 – 154°C; $[\alpha]_D$ +14.8° (c 0.68, $CHCl_3$); cd λ max (MeOH) nm ($\Delta\varepsilon$), 267 (-1.1), 240 (+1.6), 228 (+1.1), 210 (-1.9); ms m/z (%), 851 (M^+, 28, calcd, $C_{43}H_{49}NO_{17}$: 851.3001; found : 851.2996), 792 (29), 734 (36), 218 (5), 206 (16), 178 (25), 105 (100); ir ν max ($CHCl_3$) cm^{-1}, 1758 (br s), 1602 (s), 1585 (s), 1571 (s); uv λ max (MeOH) nm (log ε), 202 (4.25), 228 (4.24), 267 (3.59); ^{1}H-nmr ($CDCl_3$, 400 MHz), listed in Table 2; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

4-Deoxyeuonymine (7) - Colorless amorphous solid; mp, 138 – 142°C; $[\alpha]_D$ -13.6° (c 1.41, $CHCl_3$); cd λ max (MeOH) nm ($\Delta\varepsilon$), 273 (+0.8), 243 (+2.0), 230 (+2.5), 208 (-2.4); ms m/z (%), 789 (M^+, 52, calcd, $C_{38}H_{47}NO_{17}$: 789.2844; found : 789.2796), 730 (35), 686 (18), 368 (12), 259 (17), 206 (51), 107 (100); ir ν max ($CHCl_3$) cm^{-1}, 1762 (br s), 1585 (s), 1568 (s); uv λ max (MeOH) nm (log ε), 202 (3.93), 213 (3.78), 263 (3.43); ^{1}H-nmr ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-nmr ($CDCl_3$, 100 MHz), listed in Table 3.

REFERENCES

1. H. Itokawa. K. Takeya, K. Watanabe, H. Morita, Y. Ichihara, N. Totsuka, O. Shirota, H. Izumi, M. Satake, I. Yasuda, U. Sankawa, M. Motidome, and F. A. Flores, J. Pharmacobio-Dyn., 1992, **15**, s-2.
2. H. Itokawa, O. Shirota, H. Morita, K. Takeya, N. Tomioka, and A. Itai, Tetrahedron Lett., 1990, **31**, 6881.
3. H. Itokawa, O. Shirota, H. Ikuta, H. Morita, K. Takeya, and Y. Iitaka, Phytochemistry, 1991, **30**, 3713.
4. H. Itokawa, O. Shirota, H. Morita, and K. Takeya, Heterocycles, 1992, **34**, 885.
5. H. Itokawa, O. Shirota, H. Morita, K. Takeya, and Y. Iitaka, J. Chem. Soc., Perkin Trans. 1, 1993, 1247.
6. H. Itokawa, O. Shirota, K. Ichitsuka, H. Morita, and K. Takeya, J. Nat. Prod., 1993, **56**, 1479.
7. O. Shirota, H. Morita, K. Takeya, and H. Itokawa, Heterocycles, 1994, **38**, 383.
8. F. A. Flores, 'Advances in Economic Botany', Vol. 1, The New York Botanical Garden, 1984, pp. 1 - 8.

9. J. G. Gonzalez, G. D. Monache, F. D. Monache, and G. B. Marini-Bettolo, J. Ethnophamacology, 1982, **5**, 73.

10. S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger, and R. F. Bryan, J. Am. Chem. Soc., 1972, **94**, 1354.

11. K. Nakanishi, V. P. Gullo, I. Miura, T. R. Govindachari, and N. Visivanathan, J. Am. Chem. Soc., 1973, **95**, 6473.

12. K. Ujita, T. Fujita, Y. Takaishi, H. Tokuda, S. Nishino, and A. Iwashima, The 39th Annual Meeting of the Japanese Society of Pharmacognosy (1992, Tokyo), Abstract Papers, p. 58.

13. F. D. Monache, G. B. Marini - Bettolo, and E. A. Bernays, Angew. Entomol., 1984, **97**, 406.

14. Y. L. Zheng, Y. Xu, and J. F. Lin, Acta Pharmaceutica Sinica, 1989, **24**, 568.

15. P. Martinod, A. Paredes, F. D. Monache, and G. B. Marini - Bettolo, Phytochemistry, 1976, **15**, 562.

16. H. Wada, Y. Shizuri, K. Yamada, and Y. Hirata, Tetrahedron Lett., 1971, 2655.

17. K. Sugiura, Y. Shizuri, H. Wada, K. Yamada, and Y. Hirata, Tetrahedron Lett., 1971, 2733.

18. Y. Shizuri, H. Wada, K. Sugiura, K. Yamada, and Y. Hirata, Tetrahedron, 1973, **29**, 1773.

19. M. Beroza, J. Org. Chem., 1963, **28**, 3562.

20. Y. Shizuri, K. Yamada, and Y. Hirata, Tetrahedron Lett., 1973, 741.

21. K. Yamada, Y. Shizuri, and Y. Hirata, Tetrahedron, 1978, **34**, 1915.

22. Goodlett, W. V. Anal. Chem., 1965, **37**, 431.

Received, 31st May, 1994