

SESQUITERPENE PYRIDINE ALKALOIDS FROM MAYTENUS CHUCHUHUASCA

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

In our previous studies concerned with sesquiterpene pyridine alkaloids, we have reported isolations from Paraguayan medicinal plant "cangorosa" (M. ilicifolia)<sup>7</sup> and Peruvian medicinal plant "chuchuhuasi" (M. ebenifolia),<sup>4,5</sup> 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.),<sup>9,15</sup> which are used for the treatment of skin cancer. From this plant, fourteen sesquiterpene pyridine alkaloids (thirteen evoninate types<sup>16-18</sup> and one wilfordate type<sup>19-21</sup>) 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),<sup>4,5</sup> 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 (mpc), 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%).

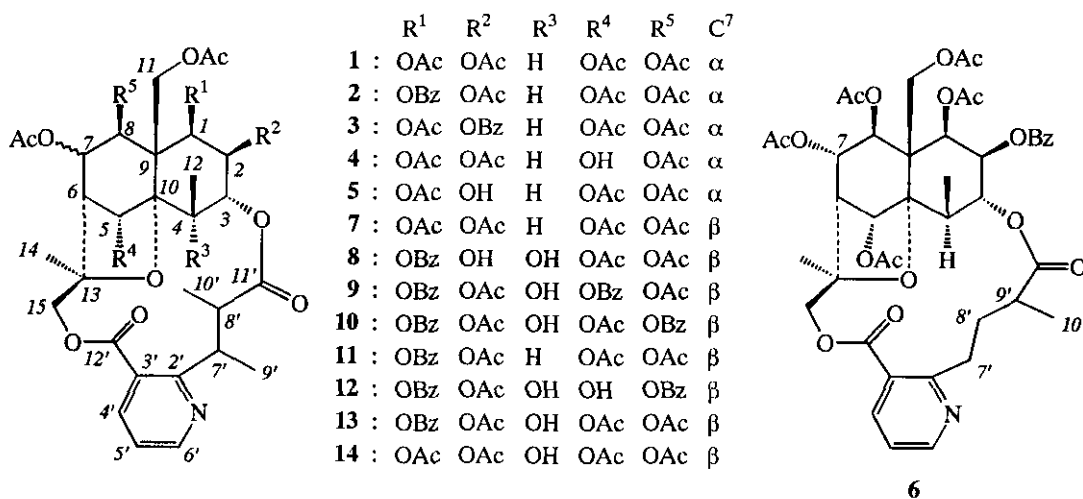


Figure 1. Structures of sesquiterpene pyridine alkaloids isolated from *M. chuchuhuasca*.

Chuchuhuanine E-I (**1**) was obtained as colorless plates with a molecular formula,  $C_{38}H_{47}NO_{17}$ . It contained six acetyl groups [ $\delta_H$ : 1.82, 1.93, 1.96, 2.09, 2.18, 2.25 (each s)], one secondary methyl group [ $\delta_H$ : 1.20 (d,  $J = 7.9$  Hz)], one tertiary methyl group [ $\delta_H$ : 1.54 (s)], two sets of methylene protons [ $\delta_H$ : 3.87, 5.21 (each d,  $J = 11.1$  Hz); 4.63, 4.66 (each d,  $J = 12.9$  Hz)], eight methine protons [ $\delta_H$ : 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- $\beta$ -agarofuran. It also contained one 2, 3-disubstituted pyridine unit [ $\delta_H$ : 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 [ $\delta_H$ : 1.24 (d,  $J = 7.4$  Hz), 1.38 (d,  $J = 6.9$  Hz)] and two methine protons [ $\delta_H$ : 2.75 (q,  $J = 7.4$  Hz), 4.15 (q,  $J = 6.9$  Hz)] which are coupling each other, corresponding to an evoninic acid.<sup>16-18</sup> 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<sup>16-18</sup> as a dihydro- $\beta$ -agarofuran sesquiterpene core, the orientations of ester groups are  $1\beta_{eq}$ ,  $2\beta_{ax}$ ,  $5\alpha_{eq}$ ,  $7\beta_{ax}$  and  $8\beta_{eq}$ , 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  $\alpha$  equatorial orientation. Therefore, the structure of chuchuhuanine E-I was determined as **1**.

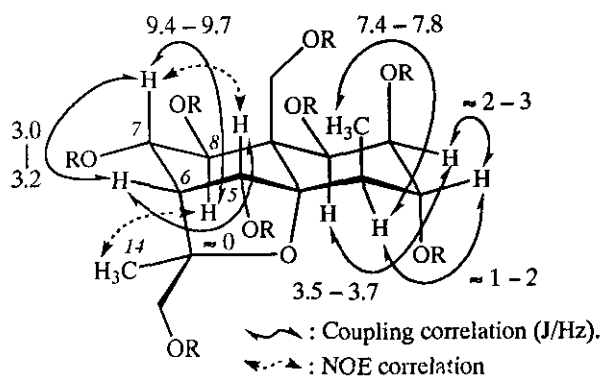


Figure 2. Configuration of a sesquiterpene core on chuchuhuanines E-I (**1**) - E-V (**5**), and W-I (**6**).

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 [ $\delta_H$ : 1.40, 1.92, 2.12; 2.22, 2.30 (each s)] and one benzoyl group [ $\delta_H$ : 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  $7\alpha$ eq 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 ( $\delta_H$ : 1.40), and was determined to be at C-1 as shown in Figure 3 by COLOC spectrum. That is, the carbonyl carbon at  $\delta_C$  164.41 gave cross peaks with the methine proton ( $\delta_H$ : 6.02) of position 1 and the ortho protons ( $\delta_H$ : 7.83) of benzoyl group. These data showed that the structure of chuchuhuanine E-II (**2**) was as shown in Figure 1.

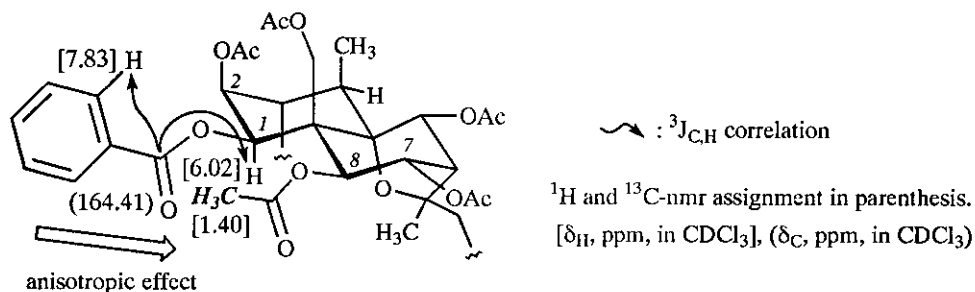


Figure 3. Partial structure of chuchuhuanine E-II (**2**).

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  $\delta_C$  165.06, and H-2 methine proton ( $\delta_H$  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. <sup>1</sup>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 <sub>3</sub>	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 <sub>3</sub>	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 <sub>3</sub>	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 <sub>3</sub>	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)	1.40 (s)	1.82 (s)	1.83 (s)	1.88 (s)	1.84 (s)
	1.93 (s)	1.92 (s)	1.94 (s)	1.90 (s)	1.93 (s)	1.95 (s)
	1.96 (s)	2.12 (s)	1.99 (s)	1.94 (s)	1.95 (s)	2.10 (s)
	2.09 (s)	2.22 (s)	2.21 (s)	2.09 (s)	2.16 (s)	2.13 (s)
	2.18 (s)	2.30 (s)	2.30 (s)	2.14 (s)	2.22 (s)	2.17 (s)
	2.25 (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<sub>3</sub> at 400 MHz. Multiplicity and coupling constants (J/Hz) given in parenthesis.

the H-5 methine proton ( $\delta_H$ : 5.06, d,  $J = 4.8$  Hz) was resonated at higher field than the corresponding proton of **1** and was shifted to lower field ( $\Delta$  1.4 ppm) by the addition of TAI (trichloroacetyl isocyanate) reagent.<sup>22</sup> 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 7 $\alpha$ cq type skeleton but 4-deoxyeuonyminol with 7 $\beta$ ax type skeleton. Therefore the structure of compound **7** was assigned to be 4-deoxyeuonymine.

Table 2.  $^1H$ -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 <sub>3</sub>	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 <sub>3</sub>	1.30 (d, 7.9)		2.17 (s)
14-CH <sub>3</sub>	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<sub>3</sub> at 400 MHz.

Multiplicity and coupling constants (J/Hz) given in parenthesis.

Table 3.  $^{13}\text{C}$ -Nmr chemical shifts (ppm) for chuchuhuanines E-I - V (1 - 5), W-I (6), and 4-deoxyuonimine (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)	168.16 (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 <sub>3</sub> )	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  $\text{CDCl}_3$  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,<sup>19-21</sup> which was representative with one secondary methyl group [ $\delta_H$ : 1.15 (d,  $J = 7.1$  Hz)], one methine proton [ $\delta_H$ : 2.35 (m)] and two sets of methylenes [ $\delta_H$ : 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 7 $\alpha$ eq 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.<sup>4-5</sup>

#### 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  $cm^2 g^{-1}$ . 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 (mple) was performed with a CIG column system (22 mm i.d.  $\times$  300 mm, Kusano Scientific Co., Tokyo) packed with 10  $\mu$ m Si gel or 20  $\mu$ m ODS. Hplc was performed with an Inertsil PREP-ODS column (20 mm i.d.  $\times$  250 mm, GL Science Inc.) packed with 10  $\mu$ m ODS. Tlc was conducted on precoated Kieselgel 60 F<sub>254</sub> (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<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. The CH<sub>2</sub>Cl<sub>2</sub>-soluble fraction (155 g) was subjected to silica gel cc using an CH<sub>2</sub>Cl<sub>2</sub>/ 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 mpls 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<sub>2</sub>O or acetonitrile / H<sub>2</sub>O solvent systems.

Chuchuhuanine E-I (1) - Colorless plates; mp, 247 - 250°C, [ $\alpha$ ]<sub>D</sub> -23.3° (c 0.77, CHCl<sub>3</sub>); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 275.9 (+0.8), 257 (-0.1), 242 (+3.2), 229 (+4.0); ms m/z (%), 789 (M<sup>+</sup>, 29, calcd, C<sub>38</sub>H<sub>47</sub>NO<sub>17</sub> : 789.2844; found : 789.2834), 730 (31), 686 (7), 259 (13), 229 (10), 206 (38), 178 (24), 134 (28), 107 (100); ir  $\nu$  max (CHCl<sub>3</sub>) cm<sup>-1</sup>, 1768 (br s), 1585 (s), 1568(s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 202 (4.02), 212 (3.95), 261 (3.64); <sup>1</sup>H-nmr (CDCl<sub>3</sub>, 400 MHz), listed in Table 1; <sup>13</sup>C-nmr (CDCl<sub>3</sub>, 100 MHz), listed in Table 3.

Chuchuhuanine E-II (2) - Colorless amorphous solid; mp, 158 - 160°C; [ $\alpha$ ]<sub>D</sub> -8.5° (c 0.46, CHCl<sub>3</sub>); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 245 (+7.8), 222 (-6.7); ms m/z (%), 851 (M<sup>+</sup>, 12, calcd, C<sub>43</sub>H<sub>49</sub>NO<sub>17</sub> : 851.3001; found : 851.3014), 792 (10), 381 (7), 259 (10), 218 (21), 206 (27), 178 (29), 134 (31), 105 (100); ir  $\nu$  max (CHCl<sub>3</sub>) cm<sup>-1</sup>, 1762 (br s), 1603 (s), 1585 (s), 1568 (s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 202 (4.25), 228 (4.28), 261 (3.63); <sup>1</sup>H-nmr (CDCl<sub>3</sub>, 400 MHz), listed in Table 1; <sup>13</sup>C-nmr (CDCl<sub>3</sub>, 100 MHz), listed in Table 3.

Chuchuhuanine E-III (3) - Colorless amorphous solid; mp, 156 - 160°C, [ $\alpha$ ]<sub>D</sub> -412.5° (c 0.04, CHCl<sub>3</sub>); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 274 (+0.8), 259 (-0.6), 229 (+8.8), 211 (-0.7); ms m/z (%), 851 (M<sup>+</sup>, 6, calcd, C<sub>43</sub>H<sub>49</sub>NO<sub>17</sub> : 851.3001; found : 851.3022), 792 (7), 367 (3), 269 (6), 219 (9), 206 (12), 178 (13), 169 (21), 105 (100); ir  $\nu$  max (CHCl<sub>3</sub>) cm<sup>-1</sup>, 1751 (br s), 1602 (s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 203 (4.18), 229 (4.31), 263 (3.65); <sup>1</sup>H-nmr (CDCl<sub>3</sub>, 400 MHz), listed in Table 1; <sup>13</sup>C-nmr (CDCl<sub>3</sub>, 100 MHz), listed in Table 3.

Chuchuhuanine E-IV (4) - Colorless amorphous solid; mp, 162 - 166°C; [ $\alpha$ ]<sub>D</sub> -9.3° (c 0.51, CHCl<sub>3</sub>); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 268 (+2.3), 232 (+6.6), 211 (-3.1); ms m/z (%), 747 (M<sup>+</sup>, 77, calcd, C<sub>36</sub>H<sub>45</sub>NO<sub>16</sub> : 747.2738; found : 747.2762), 674 (55), 259 (21), 206 (100), 161 (82); ir  $\nu$  max (CHCl<sub>3</sub>) cm<sup>-1</sup>, 3614 (br s), 1763 (br s), 1586 (s), 1568(s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 202 (3.91), 210 (3.86), 261 (3.52); <sup>1</sup>H-nmr (CDCl<sub>3</sub>, 400

MHz), listed in Table 1;  $^{13}\text{C}$ -nmr ( $\text{CDCl}_3$ , 100 MHz), listed in Table 3.

Chuchuhuanine E-V (5) - Colorless amorphous solid; mp, 154 – 160°C;  $[\alpha]_D$  -18.3° (c 0.45,  $\text{CHCl}_3$ ); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 274 (+0.6), 241 (+2.2), 210 (-2.7); ms  $m/z$  (%), 747 ( $\text{M}^+$ , 26, calcd,  $\text{C}_{36}\text{H}_{45}\text{NO}_{16}$  : 747.2738 ; found : 747.2721), 688 (24), 614 (16), 269 (17), 219 (30), 206 (49), 178 (38), 169 (44), 107 (100); ir  $\nu$  max ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ , 3615 (br s), 1762 (br s), 1585 (s), 1568 (s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 202 (3.87), 213 (3.76), 264 (3.43);  $^1\text{H}$ -nmr ( $\text{CDCl}_3$ , 400 MHz), listed in Table 1;  $^{13}\text{C}$ -nmr ( $\text{CDCl}_3$ , 100 MHz), listed in Table 3.

Chuchuhuanine W-I (6) - Colorless amorphous solid; mp, 149 – 154°C;  $[\alpha]_D$  +14.8° (c 0.68,  $\text{CHCl}_3$ ); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 267 (-1.1), 240 (+1.6), 228 (+1.1), 210 (-1.9); ms  $m/z$  (%), 851 ( $\text{M}^+$ , 28, calcd,  $\text{C}_{43}\text{H}_{49}\text{NO}_{17}$  : 851.3001; found : 851.2996), 792 (29), 734 (36), 218 (5), 206 (16), 178 (25), 105 (100); ir  $\nu$  max ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ , 1758 (br s), 1602 (s), 1585 (s), 1571 (s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 202 (4.25), 228 (4.24), 267 (3.59);  $^1\text{H}$ -nmr ( $\text{CDCl}_3$ , 400 MHz), listed in Table 2;  $^{13}\text{C}$ -nmr ( $\text{CDCl}_3$ , 100 MHz), listed in Table 3.

4-Deoxyeuonymine (7) - Colorless amorphous solid; mp, 138 – 142°C;  $[\alpha]_D$  -13.6° (c 1.41,  $\text{CHCl}_3$ ); cd  $\lambda$  max (MeOH) nm ( $\Delta\epsilon$ ), 273 (+0.8), 243 (+2.0), 230 (+2.5), 208 (-2.4); ms  $m/z$  (%), 789 ( $\text{M}^+$ , 52, calcd,  $\text{C}_{38}\text{H}_{47}\text{NO}_{17}$  : 789.2844; found : 789.2796), 730 (35), 686 (18), 368 (12), 259 (17), 206 (51), 107 (100); ir  $\nu$  max ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ , 1762 (br s), 1585 (s), 1568 (s); uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ), 202 (3.93), 213 (3.78), 263 (3.43);  $^1\text{H}$ -nmr ( $\text{CDCl}_3$ , 400 MHz), listed in Table 1;  $^{13}\text{C}$ -nmr ( $\text{CDCl}_3$ , 100 MHz), listed in Table 3.

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