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TWO NEW SESQUITERPENE PYRIDINE ALKALOIDS FROM *MAYTENUS CHUCHUHUASCA*

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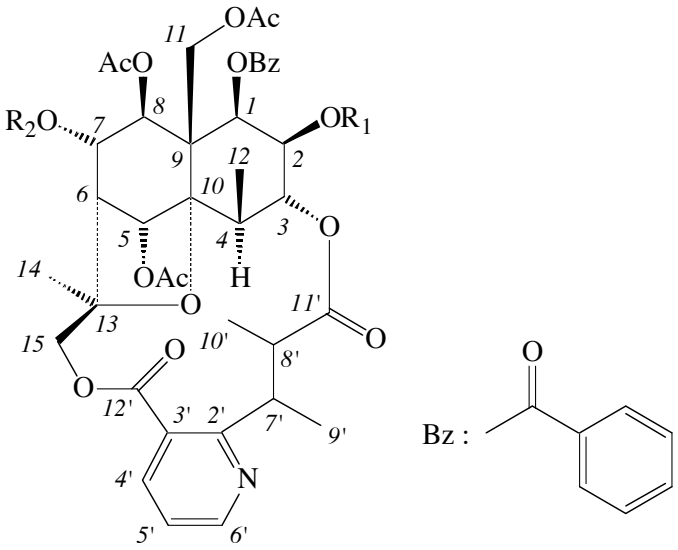
Abstract – Two new sesquiterpene pyridine alkaloids, chuchuhuanines E-VI (**1**) and E-VII (**2**), were isolated from root barks of *Maytenus chuchuhuasca* Raymond-Hamet et Colas (Celastraceae). Their structures were elucidated by the analysis of spectral data.

INTRODUCTION

Plants of the genus *Maytenus* (Celastraceae) are widely used as folk medicines in South America.[4,5] From this species many characteristic bioactive compounds, such as the maytansinoids with antitumor activity,[6] quinoid triterpenes with cytotoxic activity,[7-9] sesquiterpene polyesters with anti-tumor-promoting activity,[10] and sesquiterpene pyridine alkaloids with insect antifeedant or insecticidial activity,[11] so far have been isolated. Concerning sesquiterpene pyridine alkaloids, which possess a dihydro-β-agarofuran type sesquiterpene core with several ester moieties including a fifteen- or sixteen-membered macro cyclic diester ring system, we have previously isolated and reported several new compounds: three from a Paraguayan folk medicinal plant “cangorosa” (*M. ilicifolia* Mart.),[12] seven from a Peruvian folk medicinal plant "chuchuhuasi" (*M. ebenifolia*),[13,14] and seven from a Brazilian “xuxuá” (*M. chuchuhuasca* Raymond-Hamet et Colas).[15] We have also analyzed the conformation and evaluated the flexibility of their fifteen- or sixteen-membered macro cyclic ring system.[14] In the present study, we isolated two new additional sesquiterpene pyridine alkaloids, chuchuhuanines E-VI (**1**) and E-VII (**2**), from Brazilian medicinal plant "xuxuá" (*Maytenus chuchuhuasca* Raymond-Hamet et Colas.).[5,16] This paper describes their isolation and structure elucidation.

RESULTS AND DISCUSSION

The granddaughter fractions of Fr. V, which was one of twelve fractions came from a CH_2Cl_2-soluble portion of MeOH extract of *Maytenus chuchuhuasca*,[15,17] were further proceeded to purification. Using a preparative reversed-phase HPLC column with aqueous CH_3CN elution, Fr. V-F-23 gave chuchuhuanine E-VI (**1**), and Fr. V-F-20 gave chuchuhuanine E-VII (**2**).



chuchuhuanine E-VI (**1**) : R_1 = OBz R_2 = OAc
chuchuhuanine E-VII (**2**) : R_1 = OAc R_2 = OBz

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

Compounds (**1**) and (**2**) were obtained as colorless amorphous solid. Their FAB-MS spectra showed an identical $[M + H]^+$ ion peak at *m/z* 914. Based on HRFAB-MS analyses, their molecular formula was also found to be identical, $C_{48}H_{51}NO_{17}$. In the high field region of their ^{1}H-NMR spectra, each compound showed three doublet methyl signals, one singlet methyl signal, three methine signals, and four acetyl methyl signals, one of which was shifted to quite high field (around δ_H 1.35) by anisotropic effect. On the aromatic region, 13 methine proton signals were observed in each compound, and were assigned to be two sets of mono-substituted benzene ring and one set of 2,3-disubstituted pyridine ring by means of H-H COSY experiment. Remained proton signals were mostly oxy-methine and oxy-methylene protons, and their coupling connectivity was elucidated by H-H COSY. These data indicated that both compounds were sesquiterpene pyridine alkaloids consisted of a dihydro-β-agarofuran as a sesquiterpene and an evoninic acid as the 2,3-disubstituted pyridine contained unit, which formed one 15-membered macro cyclic structure by two intramolecular ester linkages.[18-20] Compounds belonging to sesquiterpene pyridine alkaloid vary their structure diversity by ester substitutions on the dihydro-β-agarofuran core. Both **1** and **2** have a identical molecular formula with four acetyl groups and two benzoyl groups in each molecule, so that their esterification pattern should be different each other. Assignment of all ester group positions for **1** and **2** was done by HMBC experiment, in which oxy-methine or oxy-methylene protons gave long-range correlations to corresponding carbonyl carbons of ester groups, and then the carbonyl carbons had correlations with those of acetyl methyl protons or benzoyl *ortho* protons, shown as follows: δ_H 6.17 (H-1)/δ_C 164.5/δ_H 7.55 (Bz_{ortho}), δ_H 5.74 (H-2)/δ_C 165.0/δ_H 8.05 (Bz_{ortho}), δ_H 6.42 (H-5)/δ_C 169.4/δ_H 2.23 (Ac), δ_H 5.62 (H-7)/δ_C 169.8/δ_H 1.94 (Ac), δ_H 5.81 (H-8)/δ_C 169.6/δ_H 1.35 (Ac), δ_H 4.86 and 5.19 (H-11)/δ_C

170.3/δ_H 2.33 (Ac) for **1**; δ_H 6.10 (H-1)/δ_C 164.4/δ_H 7.85 (Bz_{ortho}), δ_H 5.77 (H-7)/δ_C 165.4/δ_H 7.87 (Bz_{ortho}), δ_H 5.51 (H-2)/δ_C 168.6/δ_H 2.14 (Ac), δ_H 6.49 (H-5)/δ_C 169.4/δ_H 2.24 (Ac), δ_H 5.99 (H-8)/δ_C 169.7/δ_H 1.32 (Ac), δ_H 4.91 and 4.96 (H-11)/δ_C 170.2/δ_H 2.36 (Ac) for **2**. These correlations disclosed all ester group attached positions: C-1 and C-2 to be benzoyl group, C-5, 7, 8, and C-11 to be acetyl group for **1**; whereas C-1 and C-7 to be benzoyl group, C-2, 5, 8, and C-11 to be acetyl group for **2**, respectively. The HMBC also confirmed the formations of dihydro-β-agarofuran skeleton, evoninic acid moiety, and their diester linkage at C-3 and C-15. Generally, most of sesquiterpene pyridine alkaloids possess an euonyminol as their dihydro-β-agarofuran core, which ester group orientations are 1βeq, 2βax, 5αeq, 7βax and 8βeq, and containing a hydroxyl group at C-4.[15-17] However, the dihydro-β-agarofuran core of both **1** and **2** was obviously different from euonyminol as follows. First, C-4 hydroxyl group was gone and an alternative methine hydrogen was observed in ^{1}H-NMR spectrum. Second, coupling constants among H-6, H-7, and H-8 methine protons does not match with those of sesquiterpene pyridine alkaloids based on euonyminol. In the euonyminol skeleton, their coupling constants are generally $J_{6,7}$ = 3.8 to 4.0 Hz and $J_{7,8}$ = 5.6 to 5.9 Hz, whereas **1** showed $J_{6,7}$ = 3.3 Hz and $J_{7,8}$ = 9.5 Hz, **2** showed $J_{6,7}$ = 3.1 Hz and $J_{7,8}$ = 9.8 Hz for the couplings among H-6, H-7 and H-8 (Figure 2). This indicated that C-7 ester group was αeq orientation in both **1** and **2**, so that the dihydro-β-agarofuran core of them was 4-desoxy-isoeuonyminol.[14] ROESY experiment for **1** and **2** also supported this conclusion (Figure 2). Therefore, the structure of chuchuhuanines E-VI (**1**) and E-VII (**2**) was decided as shown in Figure 1.

Complete ^{1}H- and ^{13}C-NMR signals assignments of chuchuhuanines E-VI (**1**) and E-VII (**2**), which were done using HSQC and HMBC interpretations, are shown in Table 1.

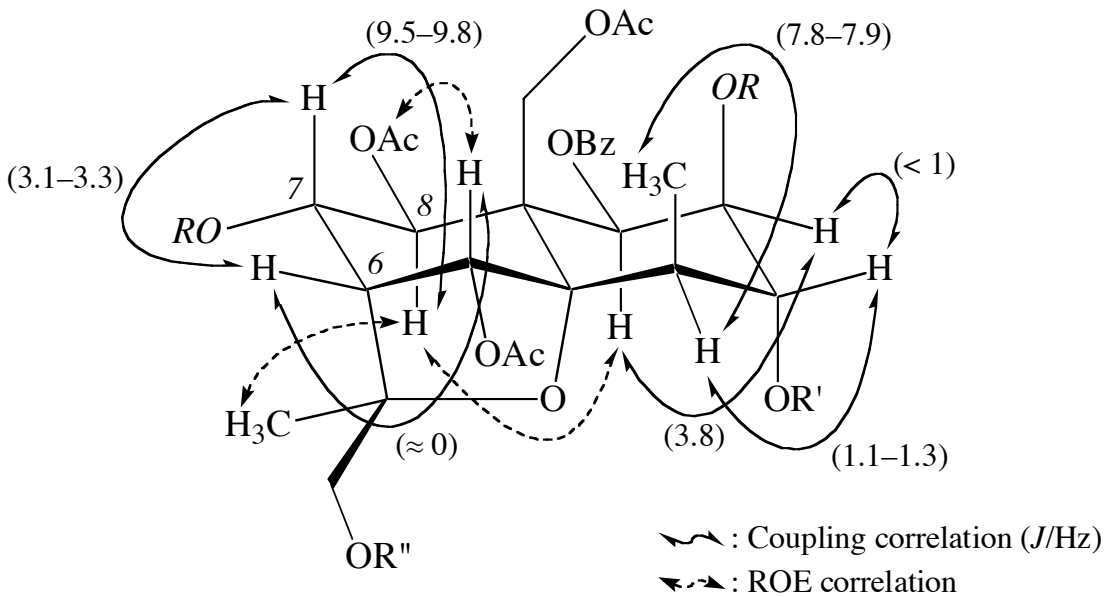


Figure 2 Configuration of a sesuqiterpene core on chuchuhuanines E-VI (**1**) and E-VII (**2**).

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectral data for **1** and **2** in $CDCl_3$.

Position	chuchuhuanine E-VI (**1**)		chuchuhuanine E-VII (**2**)	
	δ_C	δ_H[a]	δ_C	δ_H[a]
1	73.1	6.17 (*d*, 3.8)	73.4	6.10 (*d*, 3.8)
2	70.3	5.74 (*br s*)	69.7	5.51 (*br s*)
3	73.4	5.00 (*br s*)	73.3	4.88 (*br s*)
4	35.7	2.85 (*dq*, 1.1, 7.8)	35.8	2.84 (*dq*, 1.3, 7.9)
5	75.1	6.42 (*s*)	75.1	6.49 (*s*)
6	49.4	2.55 (*d*, 3.3)	49.3	2.74 (*d*, 3.1)
7	73.5	5.62 (*dd*, 3.3, 9.5)	74.3	5.77 (*dd*, 3.1, 9.8)
8	74.4	5.81 (*d*, 9.5)	74.2	5.99 (*d*, 9.8)
9	50.3		50.4	
10	90.9		91.0	
11	60.9	4.86 (*d*, 13.0)	60.2	4.91 (*d*, 13.0)
		5.19 (*d*, 13.0)		4.96 (*d*, 13.0)
12	15.1	1.34 (*d*, 7.8)	14.9	1.31 (*d*, 7.9)
13	84.0		83.9	
14	19.2	1.63 (*s*)	19.3	1.70 (*s*)
15	69.7	4.00 (*d*, 11.0)	69.6	3.96 (*d*, 11.2)
		5.23 (*d*, 11.0)		5.24 (*d*, 11.2)
2’	163.9		163.8	
3’	126.5		126.5	
4’	138.0	8.02 (*dd*, 1.8, 7.8)	138.0	7.97 (*dd*, 1.8, 7.9)
5’	121.1	7.25 (*dd*, 4.8, 7.8)	121.0	7.23 (*dd*, 4.8, 7.9)
6’	151.1	8.70 (*dd*, 1.8, 4.8)	151.1	8.69 (*dd*, 1.8, 4.8)
7’	37.3	4.21 (*q*, 7.0)	37.2	4.20 (d*q*, 1.1, 7.0)
8’	43.7	2.89 (*dq*, 0.9, 7.3)	43.7	2.87 (*dq*, 1.1, 7.3)
9’	12.6	1.45 (*d*, 7.0)	12.6	1.45 (*d*, 7.0)
10’	10.5	1.35 (*d*, 7.3)	10.5	1.34 (*d*, 7.3)
11’	175.6		175.6	
12’	167.8		167.7	
1-O*C*=O	164.5		164.4	
C (*ipso*)	129.6		129.0	
*C*H (*ortho*)	129.4	7.75 (*dd*, 1.2, 8.2)	129.3	7.85 (*dt*, 1.6, 7.9)
*C*H (*meta*)	128.4	7.31 (*t*-like, 7.7)	128.5	7.40 (*dt*, 1.6, 7.9)
*C*H (*para*)	133.1	7.49 (*t*-like, 7.5)	133.3	7.51 (*m*)
2-OC=O	165.0		168.6	
CH_3			20.9	2.14 (*s*)
C (*ipso*)	128.8			
*C*H (*ortho*)	129.9	8.05 (*dd*, 1.1, 8.2)		
*C*H (*meta*)	128.8	7.51 (*t*-like, 7.6)		
*C*H (*para*)	133.8	7.62 (*t*-like, 7.5)		
5-O*C*=O	169.4		169.4	
CH_3	21.4	2.23 (*s*)	21.4	2.24 (*s*)
7-O*C*=O	169.8		165.4	
CH_3	20.8	1.94 (*s*)		
C (*ipso*)			129.6	
*C*H (*ortho*)			129.6	7.87 (*dt*, 1.5, 7.6)
*C*H (*meta*)			128.6	7.37 (*dd*, 1.5, 7.6)
*C*H (*para*)			133.6	7.54 (*m*)
8-O*C*=O	169.6		169.7	
CH_3	20.1	1.35 (*s*)	20.2	1.32 (*s*)
11-O*C*=O	170.3		170.2	
CH_3	21.2	2.33 (*s*)	21.3	2.36 (*s*)

[a]Multiplicity and coupling constants (*J*/Hz) were given in parenthesis.

EXPERIMENTAL

General Experimental Procedures. The experimental procedures were same as those described previously.[14] The other procedures are specified as follows: ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were recorded on a Varian Unity Plus 400 spectrometer at 300 K using Varian standard pulse sequences with standard parameters. Phase-sensitive ROESY experiments were conducted with a mixing time of 300 msec. Field gradient HSQC and HMBC experiments were performed with a 150 msec delay to optimize the one-bond correlation in HSQC spectra and suppress them in HMBC spectra, and with a 63 msec evolution delay for long-range couplings in HMBC spectra.

Plant Material. Dark reddish brown stem barks of *Maytenus chuchuhuasca* Raymond-Hamet et Colas (5 kg), commonly known as "xuxuá", were purchased in São Paulo, Brazil in 1992.[14] 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 University of Pharmacy and Life Science.

Extraction and Isolation. Crushed barks (5 kg) of *Maytenus chuchuhuasca* were extracted three times with MeOH (total 54 L) at 50–65 °C for 6 hours each time to give a MeOH extract (1.5 kg), which was partitioned between CH_2Cl_2 and H_2O.[14] The CH_2Cl_2-soluble fraction (155 g) was subjected to silica gel cc using a CH_2Cl_2—EtOAc gradient system (1:0—0:1) following MeOH to give twelve fractions (Fr. I—XII).[14] The fraction V (9.9 g) was further subjected to ODS MPLC with CH_3CN—H_2O stepwise gradient system (7.5:2.5—1:0) to give 20 daughter fractions (Fr. V-A to T). Each daughter fraction was further separated by silica gel MPLC using *n*-Hexan—EtOAc gradient system or ODS MPLC using MeOH—H_2O gradient system. One of granddaughter frandtions, Fr. V-F-23 (60 mg), was conducted on ODS-HPLC purification with 61 % aqueous CH_3CN elution gave chuchuhuanine E-VI (**1**; 19.9 mg). Another granddaughter fraction, Fr. V-F-20 (27 mg), gave chuchuhuanine E-VII (**2**; 4.5 mg) by using ODS-HPLC eluted with 62 % aqueous CH_3CN.

Chuchuhuanine E-VI (1): Colorless amorphous solid; CD λ_{max} (MeOH) nm ($\Delta\varepsilon$), 269.5 (+1.2), 239.0 (+18.4), 222.0 (-7.2); UV λ_{max} (MeOH) nm (log ε), 229.5 (4.48), 265.0 (3.69); FAB-MS *m/z* (%), 914.3 (100, $[M+H]^+$), 854.2 (7), 792.1 (10); HRFAB-MS *m/z* 914.3218 (calcd, $C_{48}H_{51}NO_{17}$,914.3235); IR ν_{max} ($CHCl_3$) cm^{-1}, 3459, 2982, 1732, 1586, 1453, 1372, 1246, 1109, 712; ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 1.

Chuchuhuanine E-VII (2): Colorless amorphous solid; CD λ_{max} (MeOH) nm ($\Delta\varepsilon$), 267.0 (+2.3), 239.0 (+22.8), 222.5 (-15.2); UV λ_{max} (MeOH) nm (log ε), 230.0 (4.49), 265.0 (3.67); FAB-MS *m/z* (%), 914.3 (100, $[M+H]^+$), 854.1 (12); HRFAB-MS *m/z* 914.3184 (calcd, $C_{48}H_{51}NO_{17}$,914.3235); IR ν_{max} ($CHCl_3$) cm^{-1}, 3449, 2982, 1757, 1586, 1453, 1372, 1219, 1111, 712; ^{1}H-NMR ($CDCl_3$, 400 MHz), listed in Table 1; ^{13}C-NMR ($CDCl_3$, 100 MHz), listed in Table 1.

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