HETEROCYCLES, Vol. 68, No. 6, 2006, pp. 1241 - 1247. © The Japan Institute of Heterocyclic Chemistry
Received, 13th March, 2006, Accepted, 10th April, 2006, Published online, 11th April, 2006. COM-06-10728

EVONINOATE SESQUITERPENE ALKALOIDS FROM THE STEM OF *CELASTRUS PANICULATUS*

Yasu Lu,[1] Shilin Yang,[2] Zhongmei Zou,[1] Xiuzhen Luo,[1] Hebin Chen,[3] and Lizhen Xu[1,*]

[1]Institute of Medicinal Plant Development, Beijing, 100094, China; [2]National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine; Jiangxi College of Traditional Chinese Medicine, Nanchang, 330006, China; [3]National Center of Biomedical Analysis, Beijing, 100850, China.

Email: xulizhen2002@hotmail.com

Abstract –Two novel evoninoate sesquiterpene alkaloids, paniculatine A (**4**) and paniculatine B (**5**) with two known, euonymin (**2**) and wifornine F (**3**), were isolated from the stem of *Celastrus paniculatus.* The structures of the new compounds were elucidated by 2D NMR techniques and spectral comparison. The configuration of C-2' and C-3' of **2** and **3** was determined for the first time by spectral comparison.

INTRODUCTION

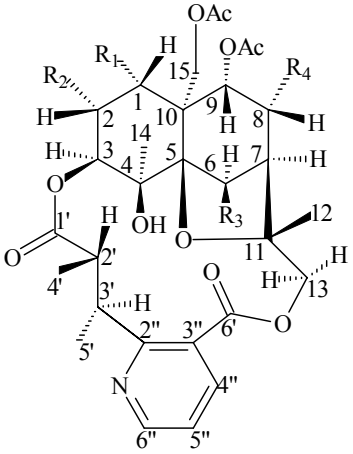
Celastrus paniculatus belongs to the Celastraceae family and widely distributed in China, which was traditionally used for the treatment of rheumatism and constipation.[1] However, few studies about the chemical constituents of *C. paniculatus* were reported. In previous investigation on the chemical constituents of Celastraceae, many sesquiterpenes were isolated, some of them have the activities of antitumor and insecticidal.[2-7] This paper reports on the isolation of two new evoninoate sesquiterpene alkaloids, paniculatine A (**4**) and B (**5**), with two known ones, euonymin (**2**)[2] and wifornine F (**3**),[3] from the stem of *C. paniculatus*. The structures of the new compounds were established by ^{1}H and ^{13}C NMR spectra and 2D NMR techniques and comparison with related compounds.

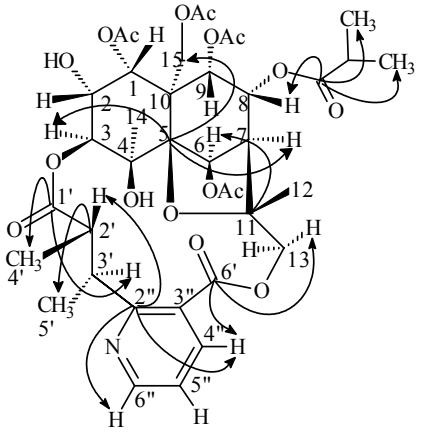
RESULTS AND DISCUSSION

Compound (**4**), white amorphous powders, gave a positive reaction to Dragendorff reagent. Its formula was determined as $C_{38}H_{49}NO_{17}$ by HRESIMS spectrum at *m/z* 792.3079 $[M+H]^+$ (calcd. 792.3092). The

IR showed OH (3448 cm^{-1}) and COO (1743 cm^{-1}) absorptions. The NMR (Table 1 and 2) spectra showed two doublet methyl groups (δ_H 1.18, 1.43 and δ_C 9.6, 11.9), two singlet methyl groups (δ_H 1.57, 1.67 and δ_C 18.5, 23.0), four acetate groups (δ_H 1.94, 1.99, 2.20, 2.30 and δ_C 20.5, 20.7, 21.2, 21.6, 168.9, 169.2, 169.8, 170.2), one isobutyrate group (δ_H 1.22, 1.24, 2.64 and δ_C 18.8, 19.2, 34.0, 176.5) and one pyridine ring (δ_H 7.32, 8.12, 8.75 and δ_C 121.4, 125.7, 137.8, 151.5, 164.8). Besides, another two ester carbonyl carbons (δ_C 168.4 and 174.2) were found in the ^{13}C NMR spectrum. From the ^1H-^1H COSY NMR spectrum, the two doublet methyl group signals (δ_H 1.18 and 1.43) were coupled to H-2' (δ_H 2.54) and H-3' (δ_H 4.68) respectively. These data suggested that **4** has β-dihydroagarofuran skeleton with an evoninoate diester bridge.

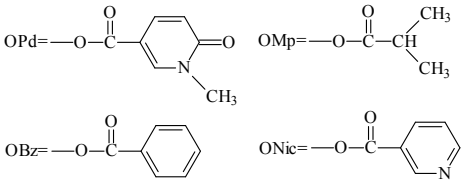
Figure 1: Structures of compounds (**1-5**) and HMBC correlations in **4**





	R_1	R_2	R_3	R_4
1	OAc	OPd	OAc	OAc
2	OAc	OAc	OAc	OAc
3	OAc	OH	OBz	OAc
4	OAc	OH	OAc	OMp
5	ONic	OAc	OBz	OAc

Key HMBC Correlations in **4**(C→H)



By comparing the NMR spectra of **4** with the known evoninoate sesquiterpene alkaloid (**3**), it was revealed that the signals of the two molecules were similar. This suggested that their nuclear structure was identical. One isobutyrate group (δ_H 1.22, 1.24, 2.64 and δ_C 18.8, 19.2, 34.0, 176.5) was found in **4**, the isobutyrate carbonyl (δ_C 176.5) was correlated with methyl (δ_H 1.22, 1.24) of isobutyrate and H-8 (δ_H 5.54) in the HMBC spectrum of **4**, indicated that the isobutyrate group was located at C-8, so that an isobutyrate group in **4** replaced the acetate group at C-8 in **3**. Four acetyl carbonyls (δ_C 168.9, 169.2, 169.8, 170.2) were correlated with H-15, H-6, H-1, and H-9 in HMBC spectrum of **4**, respectively, therefore the benzoate group at C-6 in **3** was replaced by one acetate group in **4**.

Table 1. ^{1}H NMR (600 MHz) Data for compounds (**1**-**5**) in $CDCl_3$

H		1	2	3	4	5
1		5.67 d 4.2	5.55 d 4.2	5.50 d 3.6	5.50 d 4.2	5.96 d 3.6
2		5.48 dd 4.2 2.4	5.23 dd 4.2 2.4	4.12 dd 3.6 2.4	4.01 dd 4.2 2.4	5.39 dd 3.6 2.4
3		4.78 d 2.4	4.72 d 2.4	4.80 d 2.4	4.78 d 2.4	4.82 d 2.4
6		7.04 s	7.03 s	7.17 s	7.01 s	7.14 s
7		2.38 d 4.2	2.34 d 4.2	2.51 d 3.6	2.32 d 4.2	2.56 d 4.2
8		5.54 dd 6.0 4.2	5.51 dd 6.0 4.2	5.57 dd 6.0 3.6	5.54 dd 6.0 4.2	5.54 dd 6.0 4.2
9		5.42 d 6.0	5.35 d 6.0	5.40 d 6.0	5.35 d 6.0	5.48 d 6.0
12		1.71 s	1.68 s	1.69 s	1.67 s	1.72 s
13		3.72 d 11.6	3.69 d 11.4	3.60 d 11.4	3.69 d 11.4	3.64 d 11.4
		5.98 d 11.6	5.96 d 11.4	6.04 d 11.4	5.97 d 11.4	6.06 d 11.4
14		1.57 s	1.55 s	1.60 s	1.57 s	1.62 s
15		4.16 d 13.5	4.48 d 13.8	4.71 d 13.2	4.55 d 13.2	4.67 d 13.2
		5.54 d 13.5	5.13 d 13.8	5.33 d 13.2	5.36 d 13.2	5.35 d 13.2
2'		2.57 q 6.8	2.56 q 7.2	2.56 q 7.2	2.54 q 6.6	2.63 q 7.2
3'		4.67 q 7.0	4.45 q 7.2	4.73 q 6.6	4.68 q 6.6	4.79 q 6.6
4'		1.20 d 7.0	1.20 d 7.2	1.18 d 7.2	1.18 d 6.6	1.22 d 7.2
5'		1.39 d 7.7	1.49 d 7.2	1.45 d 6.6	1.43 d 6.6	1.47 d 6.6
4"		8.06 dd 7.8 1.8	8.07 dd 7.8 1.8	8.06 dd 7.8 1.8	8.12 dd 7.8 1.8	8.08 dd 7.2 1.8
5"		7.32 dd 7.8 4.8	7.28 dd 7.8 4.8	7.28 dd 7.8 4.8	7.32 dd 7.8 4.8	7.29 dd 7.8 4.8
6"		8.70 dd 4.8 1.8	8.69 dd 4.8 1.8	8.72 dd 4.8 1.8	8.75 dd 4.8 1.8	8.73 dd 4.8 1.8
OAc-CH_3		1.81 s	1.84 s	1.97 s	1.94 s	1.54 s
		1.98 s	1.99 s	2.02 s	1.99 s	2.17 s
		2.18 s	2.15 s	2.16 s	2.20 s	2.24 s
		2.22 s	2.16 s	2.33 s	2.30 s	2.36 s
		2.38 s	2.21 s			
			2.32 s			
OMp	CH_3				1.22 d 7.2	
					1.24 d 7.2	
	CH				2.64 m	
OBz	2,6			8.33 d 7.2		8.33 d 7.2
	3,5			7.50 t 7.8		7.51 t 7.2
	4			7.59 t 7.2		7.61 t 7.2
ONic	2					9.02 br
	4					8.17 d 7.8
	5					7.39 br
	6					8.79 br

Coupling constants and NOESY experiments served to determine the relative stereochemistry of the acyloxyl and hydroxyl groups on the β-dihydroagarofuran skeleton. Generally the stereochemistry of H-1 and H-6 in this class of compounds are axial.[4,5] The coupling constant of 2.4 Hz between H-2 and H-3 indicated diequatorial relationship between them. The constant between H-7 and H-8 was 4.2 Hz,

indicating a diequatorial relationship. The constant of 6.0 Hz between H-8 and H-9 indicated a *syn* relationship, H-9 was axial. These data were identical with those of **3**. As further confirmation, the correlations between H-1 and H-2, H-1 and H-9, H-9 and H-12, H-9 and H-8 in the NOESY spectrum of **4** suggested that H-1, H-2 , H-8 and H-9 were all β, the correlations between H-6 and H-7, H-6 and C-4 methyl, C-4 methyl and H-3, H-6 and H-15 in the NOESY spectrum of **4** suggested that H-6, H-7, C-4 methyl, H-3 and C-15 were all α. Thus the relative stereochemistry of the acyloxyl and hydroxyl groups was determined as 1α, 2α, 3β, 4β, 6β, 8α and 9α. This was consistent with the most compounds of this kind.[2-8]

Emarginatine A (**1**) is an evoninoate sesquiterpene alkaloid, whose structure was previously determined by spectral and X-Ray analysis.[6] The configuration of C-2' and C-3' of compounds (**2-5**) were elucidated as shown (Figure 1) by comparing their ^{1}H and ^{13}C NMR (Table 1 and 2) spectra with those of emarginatine A, the signals of H-2', 3', 4', 5' and C-2', 3', 4', 5' were almost identical. In addition, 2-[(1*S*, 2*S*)-2-carboxy-1-methylpropyl]nicotinic acid (Figure 2) was obtained by hydrolyzing the evoninoate sesquiterpene alkaloid isolated from Celastraceae,[8] which also confirmed that the configuration of C-2' and C-3' in compounds (**2-5**) was 2'*S* and 3'*S* on the basis of biogenesis. All ^{1}H and ^{13}C signals had been designed by 2D NMR. Therefore the structure of **4** has been established as shown in figure 1, named as paniculatine A.

Figure 2: Structure of 2-[(1*S*, 2*S*)-2-carboxy-1-methylpropyl]nicotinic acid

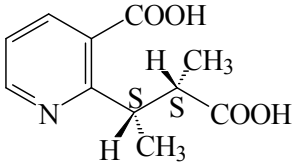


Table 2. ^{13}C NMR (150 MHz) Data for compounds (**1-5**) in $CDCl_3$

C	1	2	3	4	5
1	73.1	73.2	75.3	75.4	74.8
2	69.4	69.9	69.7	69.7	69.0
3	75.7	75.6	78.1	78.0	75.8
4	70.5	70.4	70.6	70.5	70.7
5	94.1	93.9	94.1	94.4	93.8
6	73.8	73.7	74.9	73.9	74.7
7	50.8	50.6	50.4	50.5	50.3
8	69.0	68.3	69.2	68.5	68.9
9	70.6	70.7	71.2	71.3	71.1
10	52.2	52.0	52.7	52.4	52.5
11	84.4	84.3	84.0	84.0	84.5
12	18.7	18.5	18.4	18.5	18.4

13	70.0	69.9	70.3	70.2	70.1
14	23.4	23.1	23.1	23.0	23.1
15	60.5	60.3	60.3	60.6	60.1
1'	174.0	173.9	174.2	174.2	173.7
2'	45.1	44.9	45.1	45.0	45.1
3'	36.5	36.4	36.3	36.3	36.3
4'	9.9	9.7	9.8	9.6	9.9
5'	12.0	11.8	12.0	11.9	11.9
6'	168.6	168.5	168.4	168.4	168.3
2"	165.7	165.3	165.0	164.8	165.0
3"	125.1	125.0	125.6	125.7	125.4
4"	138.0	137.8	137.6	137.8	137.8
5"	121.3	121.1	121.2	121.4	121.5
6"	151.7	151.5	151.7	151.5	151.5
OAc-CH_3	20.6	20.4	20.6	20.5	20.5
	20.7	20.4	20.8	20.7	21.1
	21.2	21.0	21.1	21.2	21.1
	21.5	21.0	21.5	21.6	21.5
	21.8	21.3			
		21.6			
OAc-C=O	169.0	168.6	169.1	168.9	168.8
	170.2	168.9	169.3	169.2	169.1
	162.7	169.0	169.9	169.8	170.1
	170.3	169.8	170.2	170.2	170.2
	171.2	170.0			
		170.2			
OMp CH_3				18.8	
				19.2	
CH				34.0	
C=O				176.5	
OBz 1			129.6		129.4
2,6			130.3		130.3
3,5			128.8		128.9
4			133.5		133.7
C=O			165.7		165.7
ONic 2					149.8
3					125.4
4					137.7
5					123.2
6					153.2
C=O					162.7

Compound (**5**), white amorphous powder, gave a positive reaction to dragendorff reagent. Its formula was determined as $C_{47}H_{50}N_2O_{18}$ based on the molecular ion peak at *m/z* 931.3092$[M+H]^+$ (calcd.

931.3137) in the HRESIMS spectrum. The NMR spectra of **5** showed the presence of four acetyl groups (δ_H 1.54, 2.17, 2.24, 2.36 and δ_C 20.5, 21.1, 21.1, 21.5, 168.8, 169.1, 170.1, 170.2), one nicotinate group (δ_H 7.39, 8.17, 8.79, 9.02 and δ_C 123.2, 125.4, 137.7, 149.8, 153.2, 162.7), and one benzoate group (δ_H 7.51, 7.61, 8.33 and δ_C 128.9, 128.9, 129.4, 130.3, 130.3, 133.7, 165.7). By comparing the NMR spectra of **5** with those of the known evoninoate sesquiterpene alkaloid (**2**), it was revealed that the two molecules were similar except that two acetates in **2** have been replaced with one nicotinate group and one benzoate group in **5**. This was consistent with the presence of EIMS fragment ions at *m/z* 105 (benzoate group) and 106 (nicotinate group). The signals of H-1, H-2, H-15, H-6 and H-7 were observed at significantly lower field than those of the corresponding protons in **2**, indicating that the nicotinate group and benzoate group were located at C-1 and C-6, respectively.[5] In the HMBC spectrum of **5**, the nicotinate carbonyl (δ_C 162.7) was correlated with H-4 (δ_H 8.17) of nicotinate group and H-1 (δ_H 5.96), so that the nicotinate group was located at C-1. While the benzoate carbonyl (δ_C 165.7) was correlated with H-2, 6 (δ_H 8.33) of benzoate group and H-6 (δ_H 7.14), indicating the benzoate group was located at C-6. The relative configurations in **5** were determined as shown in the same method as that in **4**. All ^1H and ^{13}C signals had been designed by 2D NMR. Thus the structure of **5** was determined unambiguously, named as paniculatine B.

EXPERIMENTAL

General Experimental Procedures. ^1H, ^{13}C and 2D NMR were recorded at 600 MHz on Inova-600 spectrometer. EIMS were determined on a Zabspec E instrument. Silica gel (Qingdao Haiyang, 200-300 mesh) was used for cc, and precoated silica gel (Qingdao Haiyang GF-254) plates were used for TLC. Prep HPLC was performed on a Waters liquid chromatograph using the preparative Zorbax SB-C_{18} and Alltima Silica columns. Mps were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on an Impact-400 spectrophotometer and refer to KBr pellets. UV spectra were measured on a Thermo spectrophotometer in MeOH.

Extraction and Isolation. The dried stems of *Celastrus paniculatus* (10kg) collected in 1999 at Yunnan province were powdered and extracted with EtOH. The crude EtOH extract (260g) was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated (25g) and chromatographed on silica gel (500g) eluting with petrol/EtOAc to yield 12 portions. The portion 9 was further separated by repeated RP-HPLC (ODS-C_{18}, MeOH-H_2O, 7:3) and NP-HPLC (silica gel, hexane-EtOAc, 1:4) to yield **2** (14mg), **3** (4mg), **4** (4mg) and **5** (3mg).

Compound (**4**). White amorphous powder, $C_{38}H_{49}NO_{17}$, mp 199-201℃ ($CHCl_3$); $[\alpha]_D^{25}$ -9.5° ($CHCl_3$; c 0.6); UV λ_{max}^{MeOH} nm (logε): 218(5.12), 264(2.81); IR (KBr) cm^{-1}: 3448, 2924, 2854, 1743, 1371, 1234,

1119; EIMS *m/z* 791[M^+,30], 206[100], 107[64], 71[35], 43[55]; HRESIMS *m/z* 792.3079[M+H]$^+$ (calcd. 792.3092); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2.

Compound (**5**). White amorphous powder, $C_{47}H_{50}N_2O_{18}$, mp 178-181℃ ($CHCl_3$); $[\alpha]_D^{25}$ -11.0° ($CHCl_3$; c 0.8); UV λ_{max}^{MeOH} nm (logε): 221(4.39), 266(3.83); IR (KBr) cm^{-1}: 3562, 3460, 1746, 1638, 1459, 1372, 1251; EIMS *m/z* 930[M^+,24], 206[100], 107[71], 106[35], 105[43], 43[60]; HRESIMS *m/z* 931.3092[M+H]$^+$ (calcd. 931.3137); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2.

ACKNOWLEDGEMENTS

The authors are very grateful to Professor Puzhu Cong for his help in mass spectra analysis, as well as Professor Zailin Li for collection and identification of the plant material.

REFERENCES

1. State Administration of Traditional Chinese Medicine of People's Republic of China, 'Chinese Herb,' Vol. 5, Shanghai Scientific and Technical Publishers, Shanghai, 1999, pp.174-175.
2. K. Yamada, K. Sugiura, Y. Shizuri, H. Wada, and Y. Hirata, *Tetrahedron*, 1977, **33**, 1725.
3. H. Q. Duan, Y. Takaishi, H. Momota, Y. Ohmoto, T. Takao, Y. F. Jia, and D. Li, *J. Nat. Prod.*, 2001, **64**, 582.
4. W. J. Wu, M. G. Wang, J. B. Zhu, W. M. Zhou, Z. N. Hu, and Z. Q. Ji, *J. Nat. Prod.*, 2001, **64**, 364.
5. Y. H. Kuo, C. F. Chen, L. M. Yangkuo, M. L. King, C. F. Chen, and G. H. Lee, *J. Nat. Prod.*, 1995, **58**, 1735
6. Y. H. Kuo, C. H. Chen, L. M. Yangkuo, M. L. King, T. S. Wu, S. T. Lu, I. S. Chen, D.R. Mcphail, A. T. Mcphail, and G. H. Lee, *Heterocycles*, 1989, **29**, 1465
7. M. Furukawa, M. Makion, T. Uchiyama, K. Ishimi, Y. Ichinohe, and Y. Fujimoto, *Phytochemisty*, 2002, **59**, 767.
8. R. Bruning and H. Wagner, *Phytochemisty*, 1978, **17**, 1821.