HETEROCYCLES, Vol. 71, No. 4, 2007, pp. 941 - 947. © The Japan Institute of Heterocyclic Chemistry
Received, 11th January, 2007, Accepted, 22nd February, 2007, Published online, 23rd February, 2007. COM-07-10999

FOUR NEW LIGNANS FROM *KADSURA HETEROCLITA*

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Abstract- Four new lignans, heteroclitins I-L (**1-4**), were isolated from the stems of *Kadsura heteroclita* (Roxb.) Craib. Their chemical structures were elucidated on the basis of 1D and 2D NMR.

INTRODUCTION

Kadsura heteroclita (Roxb.) Craib (Schisandraceae), a climbing vine plant mainly distributed in the southwestern part of China, has been widely used in folk medicine for a long history to treat rheumatism and tracheilts.[1, 2] Its stems have been proved to possess biological activities to promote vital energy and blood circulation, to expel wind-evil and to remove wetness-evil. Previous phytochemical investigation revealed that this plant mainly contained lignans[3, 4] and triterpenoids.[5-7] In the course of our exploration for natural products from Schisandra plants, we investigated the ether extract of the stems of *K. heteroclita.* In this report we describe the isolation and characterization of four new lignans, Heteroclitin I (**1**), Heteroclitin J (**2**), Heteroclitin K (**3**), Heteroclitin L (**4**).

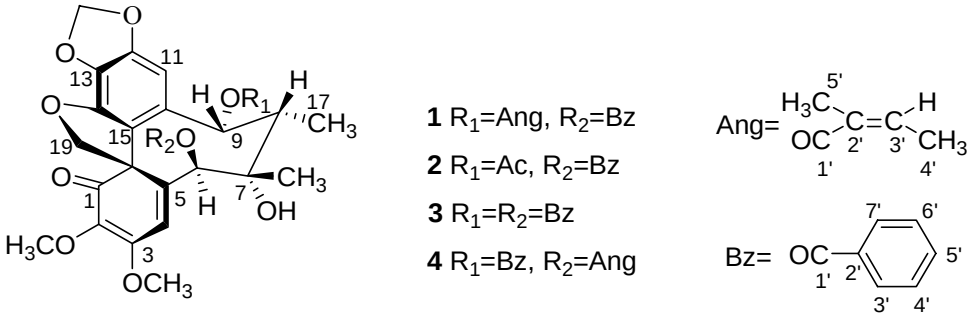

Figure 1. Structures of **1-4**

RESULTS AND DISCUSSION

Heteroclitin I (**1**), obtained as pale yellow needles, possessed molecular formula $C_{34}H_{34}O_{11}$ as determined by HREIMS (m/z 618.2107). The characteristic AB quartet signal at δ 4.61, 4.01 in the ^{1}H NMR spectrum and a quaternary carbon at δ 63.09 in the ^{13}C NMR spectrum suggested that **1** possessed a spirobenzofuranoid skeleton.[8] The CD and NMR spectra of **1** were similar to those of Kadsulignan D,[9] suggesting a close analogue. The ^{1}H NMR spectrum revealed the presence of one methyl singlet (δ 1.29, 3H s) and one secondary methyl group (δ 1.36, 3H d, J= 7.2Hz), assigned to CH_3-18 and CH_3-17, respectively; two aromatic protons (δ 6.55, 1H s; δ 6.56, 1H s); one methylenedioxy moiety (δ 5.97, 5.92 1H each, brs-like) and two methoxyl groups (δ 3.71, 3H s; δ 4.05, 3H s), located in the biphenyl rings. Moreover, the signals of a benzoate moiety (δ 7.34, 2H, d, J=7.2; δ 7.32, 2H, t, J=8; δ 7.54, 1H, t, J=7.2) and an angelate group (δ 1.75, 3H, s; δ 1.87, 3H, dd, J=7.2Hz, δ 6.01, 1H, q, J=7.2Hz) were observed in the NMR spectrum. Base on the mass spectrum, the two intense peaks at m/z 518 [M^+-C_4H_7COOH] and m/z 396 [M^+-C_4H_7COOH-C_5H_6COOH] are assigned to fragments produced by the 1, 2-elimination of benzoic acid and angelic acid *via* McLafferty ester rearrangement.[10] The HMBC spectrum of **1** displayed the correlations between H-6 (δ 5.93, 1H s) with C-1' (δ_C 165.10) of the benzoyloxy group, and H-9 (δ 5.71, 1H s) with C-1" (δ_C 165.84) of the angeloyloxy group, suggesting that the two ester groups were substituted at C-6 and C-9, respectively. According to the NOESY spectrum of 1, the correlations between H-4 with H-6α, and H-11 with H-9β, indicated the benzoyloxy and angeloyloxy groups were located at 6β and 9α, respectively. Moreover, 1 bears a twist-boat-chair conformation[11, 12] according to the NOESY correlations of Me-18 with Me-17, H-9β with Me-17, and Me-18 with H-6α (Figure 2). From the

above data, **1** should possess a similar chemical structure as Kadsulignan D except for a benzoyloxy at C-6 instead of an angeloyloxy in Kadsulignan D.

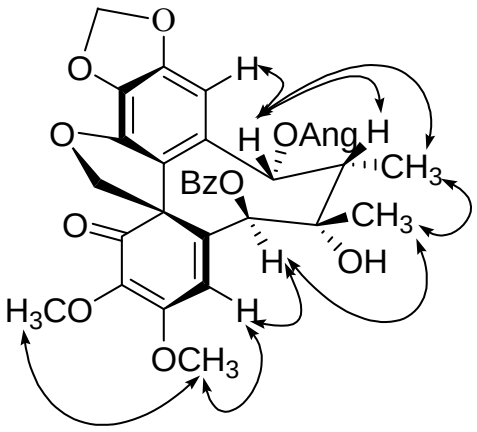

Figure 2. Key NOE correlations of **1**.

Heteroclitin J (**2**) and K (**3**) have the molecular formula $C_{31}H_{30}O_{11}$ and $C_{36}H_{32}O_{11}$, corresponding to the molecular ion peak at *m/z* 578.1777 and *m/z* 640.1945 in the HREIMS, respectively. Their IR, UV, CD and NMR spectra were similar to those of **1**, which indicated **2** and **3** were analogues of **1**. The NMR spectra demonstrated the presence of one secondary methyl, one tertiary methyl, one methylenedioxyl, two methoxyls and two aromatic protons in **2** and **3**. In addition, signals of one acetyl and one benzoyl group in **2** and two benzoyl groups in **3** were observed. The presence of these substituents is also in agreement with the EI-MS fragment peaks at *m/z* 518 [M^+-CH_3COOH] and *m/z* 396 [M^+-CH_3COOH -C_5H_6COOH] in **2** and fragment peaks at *m/z* 518 [M^+-C_5H_6COOH] and *m/z* 396 [M^+-C_5H_6COOH -C_5H_6COOH] in **3**. The HMBC spectrum of **2** revealed that the carbonyl carbon (δ 165.09) of the benzoyloxyl has correlation to H-6 (δ 5.90, 1H, s), while the carbonyl carbon (δ 168.62) of acetyloxyl group correlated to H-9 (δ 5.89, 1H, s). Thus, the location of the benzoyloxyl group is at C-6 and that of the acetyloxyl group at C-9. Based on the NOESY spectrum, the configuration of **2** and **3** was determined to be the same as that of **1**. So, the structure of **2** and **3** were defined as those shown in Figure 1.

Table 1 ^{1}H NMR(400 MHz, $CDCl_3$) Data for Compounds **1-4**

	1	**2**	**3**	**4**
4	6.55 (1H, s)	6.52 (1H, s)	6.56 (1H, s)	6.53 (1H, s)
11	6.56 (1H, s)	6.51 (1H, s)	6.64(1H, s)	6.50 (1H, s)
6	5.93 (1H, s)	5.90 (1H, s)	6.02 (1H,m)	5.79 (1H, s)
9	5.71 (1H, s)	5.89 (1H, s)	5.93 (1H, s)	5.84 (1H, s)
8	2.29 (1H,q, *J*=7.2)	2.25 (1H,q, *J*=7.2)	2.37 (1H,q, *J*=7.2)	2.23 (1H,q, *J*=7.2)
17	1.36 (3H, d, *J*=7.2)	1.33 (3H, d, *J*=7.2)	1.41 (3H, d, *J*=7.2)	1.38 (3H, d, *J*=7.2)
18	1.29 (3H, s)	1.27 (3H, s)	1.35 (3H, s)	1.32 (3H, s)
19	4.61, 4.01 (1H each, ABq, J=8.8)	4.79, 4.06 (1H each, ABq, J=8.8)	4.63, 4.02 (1H each, ABq, J=8.8)	4.63, 4.02 (1H each, ABq, J=8.8)
7-OH	2.91 (brs)	2.54 (brs)	2.95 (brs)	2.94 (brs)
2-OCH_3	3.71 (3H, s)	3.77 (3H, s)	3.16 (3H, s)	3.14 (3H, s)
3-OCH_3	4.05 (3H, s)	4.09 (3H, s)	3.90 (3H, s)	3.89 (3H, s)
OCH_2O	5.97, 5.92 (1H each, brs-like)	5.95, 5.91 (1H each, brs-like)	5.99, 5.93 (1H each, brs-like)	5.98, 5.93 (1H each, brs-like)
	Ang	Ac	Bz	Bz
	3' 6.01 (1H, dd, *J*=7.2)	2' 1.88 (3H, s)	3',7' 7.72 (2H, d, *J*=7.2)	3',7' 7.70 (2H, d, *J*=7.2)
	4' 1.87 (3H,d, *J*=7.2)		4',6' 7.30 (2H, overlapped)	4',6' 7.37 (2H, t, *J*=8)
	5' 1.75 (3H, s)		5' 7.54 (1H, t, *J*=7.2)	5' 7.54 (1H, t, *J*=7.2)
	Bz	Bz	Bz	Ang
	3",7" 7.34 (2H, d, *J*=7.2)	3",7" 7.34 (2H, d, *J*=7.2)	3",7" 7.34 (2H, overlapped)	3" 6.10 (1H, dd, *J*=7.2)
	4",6" 7.32 (2H, t, *J*=8)	4",6"7.30 (2H, t, *J*=8)	4",6" 7.30 (2H, overlapped)	4" 1.90 (3H,d, *J*=7.2)
	5" 7.54 (1H, t, *J*=7.2)	5" 7.53 (1H, t, *J*=7.2)	5" 7.54 (1H, t, *J*=7.2)	5" 1.35 (3H, s)

Heteroclitin L (**4**) has the same molecular formula $C_{34}H_{34}O_{11}$ as that of **1** based on HREIMS (m/z 618.2119). The NMR signals also suggested that **4** had a similar structure as **1**. However, from the HMBC spectrum of **4**, the correlation between C-1' (δ 166.24) and H-6 (δ 5.79, 1H, s) clearly indicated that the angelic acid ester should be located at C-6. In addition, the correlation between C-1"(δ 165.48) and H-9 (δ 5.84, 1H, s) revealed that the benzoic acid ester was located at C-9. Further NOESY experiments confirmed that **4** possessed 6β-angeloyloxyl and 9α-bezoyloxyl groups, like **1**, **2** and **3**.

The CD spectra of **1-4** showed negative Cotton effect around 320 nm and positive effect around 370 nm, which indicated that the spirobenzofuranoid skeleton has the same 16*S* configuration.[8]

EXPERIMENTAL

General Experimental Procedures. The CD spectra were recorded on a J-715 (JASCO) spectropolarimeter. Optical rotations were performed on a Perkin-Elmer digital polarimeter. Melting points were measured on a Fisher-Johns apparatus and were uncorrected.

Table 2 ^{13}C NMR(400 MHz, $CDCl_3$) Data for Compounds **1**-**4**

	1	**2**	**3**	**4**
1	194.66	196.04	194.73	194.92
2	132.31	131.88	132.09	132.01
3	155.00	155.61	154.67	154.82
4	122.84	123.05	123.91	123.84
5	141.39	141.79	140.41	140.57
6	82.37	81.46	82.14	81.97
7	75.38	75.18	75.60	75.51
8	44.15	44.13	44.35	44.15
9	82.51	82.37	83.67	83.74
10	129.73	129.24	128.72	129.26
11	100.96	101.21	101.28	101.30
12	150.28	150.16	150.24	150.20
13	130.35	130.42	130.49	130.16
14	143.43	143.43	143.55	143.57
15	120.08	120.29	120.27	119.74
16	63.09	63.25	62.97	62.95
17	18.05	17.75	18.03	18.09
18	28.28	28.41	28.57	28.61
19	77.34	77.35	79.08	77.40
20	101.94	101.98	102.00	101.94
2- OCH_3	58.77	58.94	58.97	58.69
3 OCH_3	58.55	58.89	58.55	58.47
	Ang	Ac	Bz	Bz
	1' 165.84	1' 168.62	1' 165.06	1' 165.48
	2' 141.93	2' 20.35	2' 128.72	2' 128.67
	3' 124.68		3',7' 129.43	3',7' 129.41
	4' 20.54		4',6' 128.35	4',6' 128.37
	5' 15.95		5' 133.30	5' 133.28
	Bz	Bz	Bz	Ang
	1" 165.10	1" 165.09	1" 165.52	1" 166.24
	2" 128.20	2" 128.31	2" 129.38	2" 142.17
	3",7" 129.77	3",7" 129.74	3",7"129.78	3" 125.99
	4",6" 128.31	4",6" 128.17	4",6" 128.40	4" 19.25
	5" 133.86	5" 133.88	5" 133.91	5" 15.70

UV spectra were measured on Perkin-Elmer Lambda 35 UV/VIS spectrometer. NMR spectra were measured on a Bruker AV 400 spectrometer with TMS as an internal standard. EI-MS spectra were obtained using a Micromass ZabSpec high-resolution mass spectrometer. Silica gel and silica gel GF_{254} sheets (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) were used for column chromatography and TLC, respectively.

Plant Material *Kadsura heteroclita* (Roxb.) Craib. (Schisandraceae) was collected at Sangzhi, Hunan Province, People's Republic of China, in November 2004, and identified by Prof. Si-bao Chen, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (2004KH09) has been deposited in the Herbarium of this institute.

Extraction and Isolation The dried stems of *Kadsura heteroclita* (Roxb.) Craib. (15 kg) were pulverized and extracted with 95% EtOH under reflux (2h×3). The ethanolic extract was concentrated under reduced

pressure and the concentrate mixed with 1kg of SiO_2. After evaporation of the solvent, the mixture was extracted with Et_2O in a Sohxlet apparatus. The ether extract (120g) was further separated by silica gel column chromatography by a gradient system of cyclohexane-acetone and purified repeatedly by silica gel and Sephadex LH-20 column to afford four lignans, Heteroclitin I (**1,** 968 mg), J (**2,** 8 mg), K (**3,** 92 mg) and L (**4,** 79 mg).

Heteroclitin I (**1**): Yellow needles, mp 192-194 ^oC; $[\alpha]^{20}_D$ –122.3° (*c* 1.09, MeOH); CD (MeOH) Δεnm: +33.42 (220), -38.50 (322), + 38.95 (370); UV (MeOH) λ_{max} (log ε) 219 (4.75), 275 (sh, 3.35), 325 (3.41) nm; IR (KBr) ν_{max} 3575, 2912, 1712, 1658, 1581, 1454, 1269 cm^{-1}; ^1H and ^{13}C NMR data (Table 1 and Table 2); EIMS *m/z* 618 $[M]^+$, 518, 476, 447, 396, 353, 341, 325, 313, 105, 83; HREIMS *m/z* 618.2107 $[M]^+$ (calcd for $C_{34}H_{34}O_{11}$, 618.2101).

Heteroclitin J (**2**): Yellow gum, $[\alpha]^{20}_D$ –60.5° (*c* 0.16, CH_3OH); CD (MeOH) Δεnm: +34.60 (223), -57.95 (322), + 60.04 (370); UV (MeOH) λ_{max} (log ε) 220 (4.21), 281 (3.12), 326 (3.15) nm; IR (KBr) ν_{max} 3565, 2920, 1712, 1652, 1575, 1459, 1251cm^{-1}; ^1H and ^{13}C NMR data (Table 1 and Table 2); EIMS *m/z* 578 $[M]^+$, 518, 476, 447, 396, 353, 341, 325, 313, 105, 83; HREIMS *m/z* 578.1777 $[M]^+$ (calcd for $C_{31}H_{30}O_{11}$, 578.1788).

Heteroclitin K (**3**): Yellow needles, mp 195-198 ^oC; $[\alpha]^{20}_D$ –161.7° (*c* 1.03, MeOH); CD (MeOH) Δεnm: -44.00 (220), -25.91 (320), +19.47 (370); UV (MeOH) λ_{max} (log ε) 220(4.56), 280 (3.21), 325 (3.29) nm; IR (KBr) ν_{max} 3568, 2923, 1735, 1708, 1658, 1581, 1450, 1245 cm^{-1} ; ^1H and ^{13}C NMR data (Table 1 and Table 2); EIMS *m/z* 640 $[M]^+$, 518, 476, 447, 396, 353, 341, 325, 313, 105, 83; HREIMS *m/z* 640.1952 $[M]^+$ (calcd for $C_{36}H_{32}O_{11}$, 640.1945).

Heteroclitin L (**4**): Yellow needles, mp 128-130 ^oC; $[\alpha]^{20}_D$ –28.0° (*c* 0.98, MeOH); CD (MeOH) Δεnm: +23.30 (222), -56.12 (320), +50.54 (370); UV (MeOH) λ_{max} (log ε) 221 (4.65), 278 (3.11), 327 (3.24) nm; IR (KBr) ν_{max} 3502, 2943, 1716, 1647, 1581, 1454, 1250cm^{-1}; ^1H and ^{13}C NMR data (Table 1 and Table 2); EIMS *m/z* 618 $[M]^+$, 496, 454, 425, 396, 353, 343, 325, 313, 105, 83; HREIMS *m/z* 618.2119 $[M]^+$ (calcd for $C_{34}H_{34}O_{11}$, 618.2101).

ACKNOWLEDGEMENTS

The authers thank the *National Natural Science Foundation of Peoples Republic of China* for financial support (No. 30530860).

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