Two New Limonoids from the Root Bark of Chinese Medicinal Plant *Dictamnus dasycarpus*

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Two new limonoids, kihadanin C (**1**) and 23-methoxydasylactone A (**2**), together with seven related known ones, **3**–**9**, were isolated from the root bark of the plant *Dictamnus dasycarpus*. The structures of the new compounds were elucidated on the basis of extensive analyses of their spectroscopic data (1D- and 2D-NMR, MS) and by comparison of their NMR data with those reported in the literature. To the best of our knowledge, **1** presents the first example of *A*,*D-seco* limonoid with an unusual 3,4-dihydroxy-2,5-dimethoxytetrahydrofuran moiety as ring *E*. In the bioassay *in vitro*, **7** showed moderate antibacterial activity against *Staphylococcus aureus*, while **8** and **9** displayed neuroprotective activities against H_2O_2-induced injury in SH-SY5Y cells.

Introduction. – Plants of the family Rutaceae are a rich source of limonoids, which proved to be a class of bioactive natural products with highly oxygenated and modified triterpene skeletons [1]. The unusual structural features and promising bioactive profile of limonoids have led to a growing chemical and biological interest in the [1a][2]. The plants of genus *Dictamnus*, which is composed of five species, are herbaceous perennials distributed throughout Europe and north Asia. Previous phytochemical studies of *Dictamnus* species had resulted in the isolation of limonoids, alkaloids, flavonoids, sesquiterpenoids, coumarins, and phenylpropanes, some of which exhibited various biological features such as antitumoral, antimicrobial, and anti-inflammatory activities [3].

The root bark of *Dictamnus dasycarpus* TURCZ. (Chinese name '*Bai-Xian-Pi*') is a traditional Chinese herbal medicine used for the treatment of jaundice, cough, rheumatism, and other diseases [4]. In the course of our search for bioactive compounds from Chinese medicinal plants [5], a chemical investigation of the MeOH extract of *D. dasycarpus* has resulted in the isolation of two new limonoids, named kihadanin C (**1**) and 23-methoxydasylactone A (**2**), along with seven related known compounds, including kihadanin A (**3**) [6], kihadanin B (**4**) [6], obacunone (**5**) [7], dasylactone A (**6**) [8], dasylactone B (**7**) [8], fraxinellone (**8**) [9], and isofraxinellone (**9**; *Fig. 1*) [10]. Herein, we report the isolation, structure elucidation, and bioactivity evaluation of these compounds.

[1]) These authors contributed equally to this work.

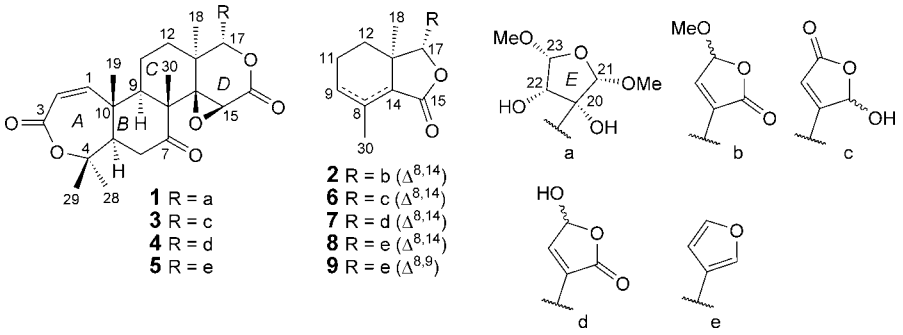

Fig. 1. *Structures of compounds* **1**–**9** *isolated from* D. dasycarpus

Results and Discussion. – The air-dried root bark of *D. dasycarpus* (2 kg) was extracted exhaustively with MeOH for three times (each for one week) at room temperature. The MeOH extract (0.2 kg) was suspended in H_2O, and then partitioned successively with petroleum ether, $CHCl_3$, and BuOH. The $CHCl_3$-soluble extract (25 g) was repeatedly chromatographed with silica gel, *Sephadex LH-20*, and *RP-C_{18}* silica gel to afford **1**–**9** (*Fig. 1*). The known compounds were readily identified as kihadanin A (**3**) [6], kihadanin B (**4**) [6], obacunone (**5**) [7], dasylactone A (**6**) [8], dasylactone B (**7**) [8], fraxinellone (**8**) [9], and isofraxinellone (**9**) [10], respectively, by comparing their spectroscopic data with those reported in the literature.

Compound **1** was obtained as optically active, white amorphous powder. The molecular formula of **1** was deduced as $C_{28}H_{38}O_{11}$ from HR-ESI-MS ($[M+Na]^+$; calc. 573.2312)) in combination with analyses of the ^{13}C-NMR and DEPT spectra. The ^{1}H-NMR spectrum of **1** (*Table*) exhibited resonances for five characteristic Me groups (δ(H) 1.18, 1.28, 1.45, 1.48, and 1.49) and one conjugated C=C bond (δ(H) 5.59 (*d*, J = 11.7, 1 H) and 6.60 (*d*, J = 11.7, 1 H)). Its ^{13}C-NMR spectrum (*Table*) displayed signals of 28 C-atoms, which were classified by DEPT and HSQC experiments as two ester C=O (δ(C) 167.0, 166.7), one ketone C=O groups (207.6), one disubstituted C=C bond (157.2, 122.9), seven Me, three sp^3 CH_2, seven sp^3 CH groups , and six sp^3 C_q atoms. These spectroscopic features were closely similar to those of co-occurring **3**–**5**, except signals of the side chain (ring *E*) attached to C(17). The ^{13}C-NMR spectrum of **1** exhibited signals of two hemiacetal C-atoms at δ(C) 108.9 (C(21)) and 112.0 (C(23)) and two O-bearing C-atoms at 81.5 (C(20)) and 76.2 (C(22)) instead of the signals of the substituted C=C bond as observed in the spectra of **3** and **4**. The ^{1}H-NMR spectrum indicated the presence of two MeO groups (δ(H) 3.37 (MeO–C(21)) and 3.47 (MeO–C(23))). The H–C(23) (δ(H) 4.96 (*d*, J = 3.6, 1 H)), which coupled with H–C(22) (δ(H) 4.15 (*d*, J = 3.6, 1 H)) in the COSY spectrum, showed HMBC cross-peaks with another hemiacetal C-atom (δ(C) 108.9 (C(21))) and the MeO group (δ(C) 56.5), location of the MeO group at C(23). The remaining MeO group (δ(C) 55.1) was placed at C(21) by its HMBC with H–C(21) (δ(H) 4.78). HMBCs H–C(21)/C(20) and H–C(22)/C(20) established the connection of C(21) and C(22) to C(20). Thus, it was concluded that the side chain at C(17) is a 20,22-dihydroxy-21,23-dimethoxytetrahydrofuran moiety. It is worth noting that such a side chain as ring *E* is very rare in the

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 125 MHz, resp.) *of* **1** *and* **2**. In $CDCl_3$; δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	6.60 (*d*, *J* = 11.7)	157.2	–	–
2	5.59 (*d*, *J* = 11.7)	122.9	–	–
3	–	167.0	–	–
4	–	84.0	–	–
5	2.59 (*dd*, *J* = 5.0, 14.1)	57.3	–	–
6	2.95 (*dd*, *J* = 14.1, 14.1),	39.9	–	–
	2.28 (*dd*, *J* = 5.0, 14.1)		–	–
7	–	207.6	–	–
8	–	53.2	–	150.8
9	2.20 (*d*, *J* = 9.8)	48.3	2.28 (*dd*, *J* = 6.6, 19.9),	32.3
			2.19 (*dd*, *J* = 7.3, 11.6)	
10	–	43.0	–	–
11	1.81 – 1.82 (*m*)	19.4	1.83 – 1.84 (*m*), 1.71 – 1.72 (*m*)	18.4
	–			–
12	2.22 – 2.25 (*m*), 1.91 – 1.92 (*m*)	30.8	2.09 – 2.10 (*m*), 1.56 – 1.58 (*m*)	32.1
				–
13	–	37.9	–	42.8
14	–	65.1	–	126.4
15	3.51 (*s*)	52.6	–	168.9
16	–	166.7	–	–
17	4.92 (*s*)	81.5	4.72 (*s*)	82.0
18	1.28 (*s*)	21.7	0.93 (*s*)	21.0
19	1.48 (*s*)	16.5	–	–
20	–	81.5	–	134.5
21	4.78 (*s*)	108.9	–	169.0
22	4.15 (*d*, *J* = 3.6)	76.2	7.23 (*s*)	145.4
23	4.96 (*d*, *J* = 3.6)	112.0	5.86 (*s*)	103.3
28	1.45 (*s*)	32.0	–	–
29	1.49 (*s*)	26.8	–	–
30	1.18 (*s*)	16.8	2.13 (*s*)	18.8
*Me*O–C(21)	3.37 (*s*)	55.1		
*Me*O–C(23)	3.47 (*s*)	56.5	3.59 (*s*)	57.4

family of naturally occurring limonoids, and, to the best of our knowledge, it has been only found in neoclerodane diterpenes, salvinicins A and B, isolated from *Salvia divinorum* (family Lamiaceae) [11], and recently reported entanosin, a tetranortriterpenoid from *Entandrophragma angolense* (family Meliaceae) [12]. The HMBC of H–C(17) to C(20)/C(21) evidenced a linkage between lactone ring *D* and the tetrahydrofuran moiety through C(17) and C(20), and further HMBC, HSQC, and COSY analysis established the constitution of **1** as depicted in *Fig. 2*.

The relative configurations at the stereogenic centers of **1** were determined to be the same as those of co-occurring **3** – **5**, based on comparison of their NMR data and biogenetic considerations. The relative configuration at the C-atoms in ring *E* was determined by NOE experiment as described in [12]. In the NOE spectrum, H–C(17) correlated with H–C(21) and H–C(22), and H–C(23) correlated with H–C(21) and

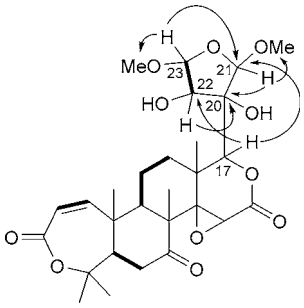

Fig. 2. *Key* $^1H,^1H$*-COSY* (—) *and HMB* (H → C) *correlations of* **1**

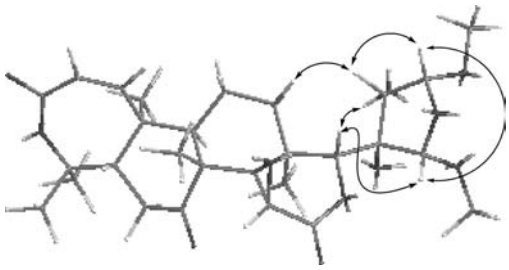

Fig. 3. *Selected NOE correlations of* **1**. Structure has been refined using MM2 force field.

H–C(22) (*Fig. 3*). These correlations could suggest that these H-atoms were on the same face of the tetrahydrofuran ring. Thus, the structure of **1** was tentatively proposed as depicted, and named kihadanin C.

Compound **2** was obtained as optically active, yellowish oil. The molecular formula of **2** was determined as $C_{15}H_{18}O_5$ by HR-ESI-MS (*m/z* 301.1050 ($[M+Na]^+$, $C_{15}H_{18}NaO_5^+$; calc. 301.1052). The ^{1}H-NMR data (*Table 1*) exhibited signals of two Me groups at δ(H) 0.93 and 2.13, and one MeO group at 3.59, and one olefin H-atom signal at 7.23. Its ^{13}C-NMR spectrum, with the aid of a DEPT experiment, displayed 15 C-atom signals, which were attributed to two ester C=O groups (δ(C) 169.0, 168.9), four olefinic C-atoms (150.8, 126.4, 134.5, and 145.4), one hemiacetal C-atom (103.3), one MeO group (57.4), one C_q-atom (42.8), one CH (82.0), three CH_2 (32.3, 32.1, and 18.4), and two Me groups (21.0, 18.8). All these spectroscopic properties of **2** were strongly reminiscent of those of the co-occurring **7**, except for the main difference by addition of one MeO group (δ(H) 3.59; δ(C) 57.4), the location of which was deduced as C(23) based on the HMBC of H–C(23) (δ(H) 5.86) to MeO–C(23) (δ(C) 57.4). In addition, the HMBC of H–C(17) (δ(H) 4.72) to C(20) (δ(C) 134.5) and C(22) (δ(C) 145.4), and the downfield chemical shift of H–C(22) (δ(H) 7.23) confirmed that the γ-hydroxybutenolide side chain was located at C(17) through C(20). The gross structure of **2** was established by further ^{1}H,^{1}H-COSY and HMBC analysis. The relative configuration of C(13) and C(17) of **2** should be the same as those of **7** on the basis of biogenetic considerations. This assumption was further supported by the lack of NOE between H–C(17) and Me(18), indicating that H–C(17) was β-oriented and Me(18) was α-oriented. Thus, the structure of **2** (*Fig. 4*) was finally established and named 23-methoxydasylactone A.

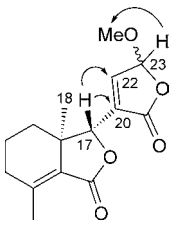

Fig. 4. *Key HMBCs* (H → C) *of* **2**

As a family of structurally interesting natural products, limonoids frequently showed attractive bioactivities. With the aim to discover novel bioactive natural products, all the isolates were tested *in vitro* for their cytotoxic, antibacterial, and neuroprotective activities. The results indicated that none of them exhibited a significant cytotoxicity against HL-60 and A549 cell lines at the concentration of 10 μM, while **6** exhibited moderate antibacterial activity against *S. aureus* with a *MIC* value of 160 g/ml. In addition, **8** and **9** displayed neuroprotective activities against H_2O_2-induced injury in SH-SY5Y cells, with 10.72 and 12.68% of increase in cell viability at 10 μM, respectively.

The isolation of the new compounds **1** and **2** in the present work has added to a diverse and complex array of the rapidly expanding family of limonoids. A literature survey indicated that naturally occurring limonoids usually possessed the common furan or γ-hydroxybutenolide moiety as ring *E*; however, limonoids possessing a 3,4-dihydroxy-2,5-dimethoxytetrahydrofuran moiety are extremely rare among natural products. To the best of our knowledge, **1** presents the first example of a limonoid possessing this fully *O*-substituted furan as side chain isolated from the Rutaceae family, and is also the first natural ring *A*,*D-seco* limonoid with such a *O*-substituted furan.

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Experimental Part

General. TLC: Precoated silica-gel plates (SiO_2; *Yantai Zifu Chemical Group Co.*, *G60*, F_{254}). Column chromatography (CC): commercial SiO_2 (*Qingdao Haiyang Chemical Group Co.*, 200–300 and 400–600 mesh) or *Sephadex LH-20* (*Amersham Pharmacial Biotech*). Reversed-phase (RP) HPLC: *Agilent 1100* series liquid chromatograph; *VWD G1314A* detector at 210 nm, semi-prep. *ZORBAX ODS* (5 μM, 9.4 × 250 mm) column (*Agilent*). Optical rotations: *PerkinElmer polarimeter 341* at the Na D-line, cell length 100 nm. ^{1}H- and ^{13}C-NMR spectra: *Varian Inova 600* (600 (^{1}H) and 125 MHz (^{13}C)); δ in ppm rel. to the solvent signal in $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) as an internal standard; *J* in Hz. Assignments supported by ^{1}H,^{1}H-COSY, HSQC, HMBC, and ROESY experiments. HR-ESI-MS: *Q-TOF micro LS-MS*/*MS* (*Waters*) mass spectrometer; in *m*/*z*.

Plant Material. The air-dried root bark of *D. dasycarpus* (2 kg) was purchased from *Shanghai Medicine Materia Corporation*, P. R. China, and identified by Prof. *Jin-Gui Shen* at the Shanghai Institute of Materia Medica, CAS. A voucher specimen (No. 12P-BXP1) has been deposited with the Herbarium of Shanghai Institute of Materia Medica, CAS.

Extraction and Isolation. The specimen was crushed into powder and extracted exhaustively with MeOH at r.t. (3×2 l), which was concentrated under reduced pressure to give a dark brown residue (0.2 kg). The dark-brown residue was then solved in H_2O and partitioned successively with hexane, $CHCl_3$, and BuOH. The $CHCl_3$ soln. was concentrated under reduced pressure to give a dark-brown residue (40 g), which was fractionated by CC (SiO_2; petroleum ether (PE)/Et_2O 99:1 to 40:60 and CH_2Cl_2/MeOH 90:10 to 50:50 to give nine fractions, *Frs. 1–9*. *Fr. 6* (2 g) was then subjected to CC (SiO_2; CH_2Cl_2/MeOH 100:1–96:4, in gradient) to yield four fractions, *Frs. 6.1–6.4*. Then, subfraction *Fr. 6.3* (1 g) was purified by CC (SiO_2; CH_2Cl_2/MeOH 90:1–19:1) to afford five fractions, *Frs. 6.3.1–6.3.5*. *Fr. 6.3.4* (0.4 g) was further purified by CC (SiO_2; CH_2Cl_2/MeOH 80:1–19:1) to give three fractions, *Frs. 6.3.4.1–6.3.4.3*. Then, *Fr. 6.3.4.2* (40.1 mg) was further purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to yield **1** (15.2 mg). *Fr. 4* (571.7 mg) was separated by CC (*Sephadex LH-20*; PE/CH_2Cl_2/MeOH 2:1:1) to afford eight fractions, *Frs. 4.1–4.8*. *Fr. 4.4* (30.0 mg) was submitted to CC (SiO_2; PE/acetone 9:1) to furnish three fractions, *Frs. 4.4.1–4.4.3*. *Fr. 4.4.1* (10.9 mg) was further purified by CC (SiO_2; PE/acetone 9:1–8:2) to yield **2** (4.5 mg). The compounds **3**–**9** were also isolated from $CHCl_3$ extract by CC (SiO_2 or *Sephadex LH-20*) successively.

Kihadanin C (= rel-*(5a*R,*5b*R,*7a*S,*8*R,*10a*S,*11a*R,*11b*R,*13a*R*)-5b,6,7,7a,8,11b,13,13a-Octahydro-8-[(2*S,*4*S,*5*R*)-tetrahydro-3,4-dihydroxy-2,5-dimethoxyfuran-3-yl]-1,1,5a,7a,11b-pentamethyloxireno[4,4a]-isochromeno[6,5-g][2]benzoxepine-3,10,12(1*H,*5a*H,*10a*H*)-trione*; **1**). White amorphous powder. $[\alpha]_D^{25} = -33.0$ ($c = 0.1$, MeOH). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table*. HR-ESI-MS: 573.1046 ($[M+Na]^+$, $C_{28}H_{38}NaO_{11}^+$; calc. 573.1052).

23-Methoxydasylactone A (= rel-*(3*R,*3a*R*)-3-(2,5-Dihydro-5-methoxy-2-oxofuran-3-yl)-3a,4,5,6-tetrahydro-3a,7-dimethyl-2-benzofuran-1(3*H*)-one*; **2**). Yellowish oil. $[\alpha]_D^{24} = +55.8$ ($c = 0.1$, MeOH). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table*. HR-ESI-MS: 301.1050 ($[M+Na]^+$, $C_{15}H_{18}NaO_5^+$; calc. 301.1052).

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