Three New Diarylheptanoids from *Myrica nana*

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Three new cyclic diarylheptanoids myricananins F–H (**1**–**3**, resp.), along with five known ones, **4**–**8**, were isolated from the roots of *Myrica nana*. Compound **3** has been obtained by *Nagai et al.* by reduction of porson with $NaBH_4$. In this work, compound **3** was isolated from natural origin for the first time. The structures of **1**–**8** were elucidated using spectroscopic methods.

Introduction. – *Myrica nana* CHEVAL. (Myricaceae) is an evergreen shrub, mainly distributed in Yunnan and Guizhou Provinces of P. R. China [1]. Its roots are used as the folk medicine for the treatment of bleeding, diarrhea, stomach pain, and skin diseases [2]. Several chemical constituents such as triterpenoids, flavonoids, tannins, and diarylheptanoids have been isolated from the bark of *Myrica* genus [3–10]. In our continuing research on cyclic diarylheptanoids of this plant [11], three new cyclic diarylheptanoids myricananins F–H (**1**–**3**, resp.) along with five known ones, **4**–**8**, have been isolated from the roots of *M. nana*. Here, we describe the isolation and structure elucidation of the new cyclic diarylheptanoids.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder. The molecular formula was established as $C_{20}H_{24}O_4$ by a *pseudo*-molecular ion in the HR-ESI-MS (positive-ion mode; at *m/z* 351.1567 ($[M+Na]^+$, calc. 351.1572)). The ^{1}H- and ^{13}C-NMR spectroscopic data (*Tables 1* and *2*, resp.) of **1** exhibited the signals of one MeO group, six CH_2 and six CH groups (including five olefinic ones), as well as seven quaternary C-atoms (all in the olefinic region). These data led us to presume that **1** is a biphenyl-type diarylheptanoid with one MeO and several OH groups. In the ^{1}H-NMR spectrum, the signals at δ(H) 6.91 (*d*, *J* = 8.3, 1 H), 7.09 (*dd*, *J* = 8.3, 2.2, 1 H), and 7.19 (*d*, *J* = 2.2, 1 H) indicated the presence of a typical *ABX* system. In addition, two *singlet*s at δ(H) 6.67 (H–C(5)) and 6.81 (H–C(19)) in the olefinic region were also observed. The ^{1}H,^{1}H-COSY spectrum indicated the correlations from CH_2(7) to CH_2(13), and from H–C(15) to H–C(16). The HMBC spectrum showed the key correlations of CH_2(7) with C(5) and C(19), CH_2(13) with C(14), H–C(18) with C(2), C(13), C(15), and C(17), H–C(19) with C(1), C(3), and C(6), MeO with C(4) (*Fig. 1*). The above evidences established the planar structure of **1** as shown. The

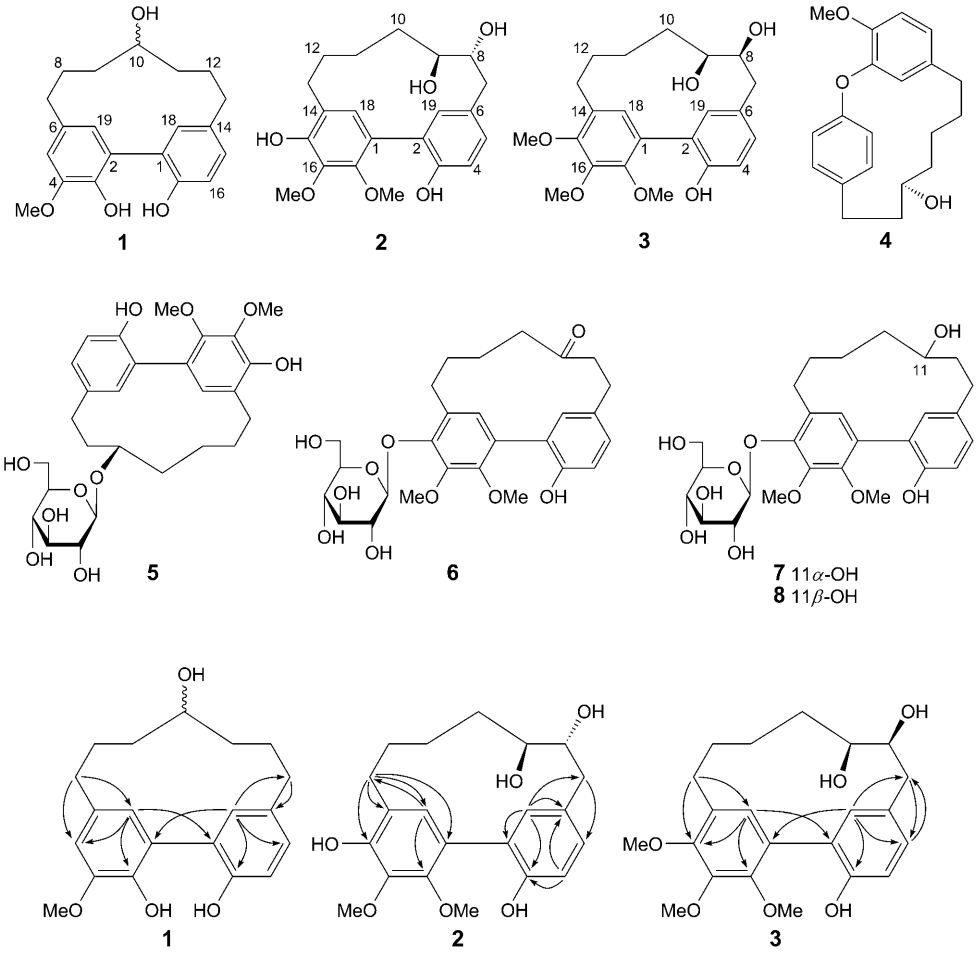

Fig. 1. *Selected HMBC of compounds* **1**, **2**, *and* **3**

preferential conformation of **1** in $CDCl_3$ was determined by a ROESY experiment, which showed interactions of H–C(5) with H_b–C(7), H–C(10) with H–C(18) and H–C(19), and H–C(19) with H_a–C(9) and H–C(18) (*Fig. 2*), suggesting that these H-atoms are spacially adjacent. However, the absolute configuration at C(10) remained unknown. Unambiguous assignments of NMR signals of **1** were performed by careful analysis of HMQC, HMBC, and ROESY experiments. Consequently, the structure of **1** was assigned as 4-methoxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2(19),3,5,14,16-hexaene-3,10,17-triol, with the trivial name myricananin F (**1**).

Compound **2** was isolated as an amorphous white powder. The molecular formula was established as $C_{21}H_{26}O_6$ by a *pseudo*-molecular-ion peak in the HR-ESI-MS (positive-ion mode; *m*/*z* 397.1611 ($[M + Na]^+$, calc. 397.1627)). The ^{13}C-NMR spectrum of **2** (*Table 2*) was similar to that of **1**, suggesting **2** to be a cyclic

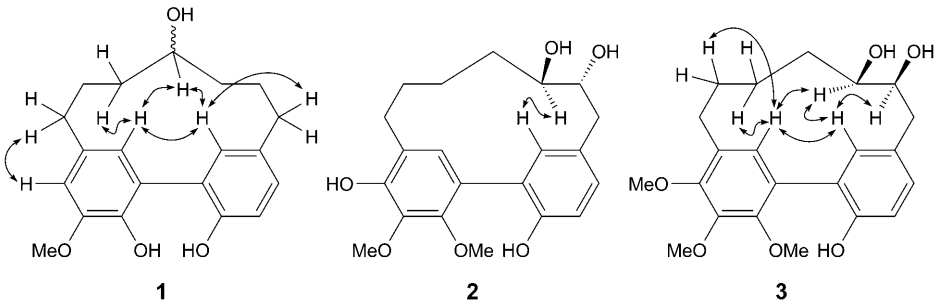

Fig. 2. *Selected ROESY correlations of compounds* **1**, **2**, *and* **3**

Table 1. *^{1}H-NMR Spectroscopic Data for Compounds* **1**–**3**[a])

Position	**1**[b])	**2**[c])	**3**[b])
4		6.75 (*d*, *J* = 8.5)	6.90 (*d*, *J* = 8.0)
5	6.67 (*s*)	6.99 (*dd*, *J* = 8.5, 2.0)	7.07 (*dd*, *J* = 8.0, 1.6)
7	2.89–2.93 (*m*, H_a),	3.01–3.06 (*m*, H_a),	3.11 (*dd*, *J* = 13.2, 3.2, H_a),
	2.48–2.55 (*m*, H_b)	3.01–3.06 (*m*, H_b)	2.88–2.95 (*m*, H_b)
8	1.82–2.01 (*m*, H_a),	3.97–3.98 (*m*)	4.33 (*dd*, *J* = 11.6, 3.6)
	1.82–2.01 (*m*, H_b)		
9	1.66–1.74 (*m*, H_a),	4.07–4.13 (*m*)	4.16 (*d*, *J* = 10.4)
	1.52–1.59 (*m*, H_b)		
10	4.12 (*t*, *J* = 9.6)	2.13–2.20 (*m*, H_a),	2.29–2.37 (*m*, H_a),
		2.13–2.20 (*m*, H_b)	1.36–1.43 (*m*, H_b)
11	1.82–2.01 (*m*, H_a),	1.95–2.04 (*m*, H_a),	1.65–1.77 (*m*, H_a),
	1.52–1.59 (*m*, H_b)	1.95–2.04 (*m*, H_b)	1.65–1.77 (*m*, H_b)
12	2.89–2.93 (*m*, H_a),	1.75 (br. *s*, H_a),	1.88–1.98 (*m*, H_a),
	2.89–2.93 (*m*, H_b)	1.75 (br. *s*, H_b)	1.88–1.98 (*m*, H_b)
13	2.28–2.36 (*m*, H_a),	2.57–2.68 (*m*, H_a),	3.83 (*s*, H_a),
	1.66–1.74 (*m*, H_b)	2.57–2.68 (*m*, H_b)	2.51–2.59 (*m*, H_b)
15	7.09 (*dd*, *J* = 8.3, 2.2)		
16	6.91 (*d*, *J* = 8.3)		
18	7.19 (*d*, *J* = 2.2)	6.97 (*s*)	6.82 (*s*)
19	6.81 (*s*)	7.72 (*d*, *J* = 2.0)	7.00 (*d*, *J* = 1.6)
MeO–C(4)	3.92 (*s*)		
MeO–C(15)			3.92 (*s*)
MeO–C(16)		3.87 (*s*)	3.97 (*s*)
MeO–C(17)		3.88 (*s*)	3.91 (*s*)

[a]) ^{1}H-NMR Data of **1** and **3** at 400 MHz, of **2** at 500 MHz. [b]) Measured in $CDCl_3$. [c]) Measured in (D_6)acetone.

diarylheptanoid. The signals at δ(H) 6.75 (*d*, *J* = 8.5, 1 H), 6.99 (*dd*, *J* = 8.5, 2.0, 1 H), and 7.72 (*d*, *J* = 2.0, 1 H) in the ^{1}H-NMR spectrum indicated the presence of a typical *ABX* system. ^{1}H,^{1}H-COSY spectrum implied two spin systems, which were CH_2(7) to CH_2(13) and H–C(4) to H–C(5). The substitution pattern of two phenyl moieties and the linkage of the aliphatic chain with the aromatic rings were established with the aid of HMBC. The HMBC spectrum of **2** showed the following key correlations (*Fig. 1*):

Table 2. *^{13}C-NMR Spectroscopic Data for Compounds* **1**–**3**[a])

Position	**1**[b])	**2**[c])	**3**[b])
1	124.4 (*s*)	124.5 (*s*)	127.0 (*s*)
2	124.8 (*s*)	123.4 (*s*)	124.8 (*s*)
3	138.9 (*s*)	152.8 (*s*)	151.9 (*s*)
4	146.6 (*s*)	116.6 (*d*)	117.1 (*d*)
5	110.1 (*d*)	130.7 (*d*)	130.1 (*d*)
6	131.4 (*s*)	126.1 (*s*)	128.9 (*s*)
7	30.4 (*t*)	38.1 (*t*)	36.1 (*t*)
8	26.6 (*t*)	77.7 (*d*)	70.2 (*d*)
9	22.8 (*t*)	73.0 (*d*)	68.8 (*d*)
10	68.7 (*d*)	36.2 (*t*)	34.7 (*t*)
11	39.5 (*t*)	25.5 (*t*)	22.5 (*t*)
12	26.9 (*t*)	26.3 (*t*)	25.9 (*t*)
13	34.8 (*t*)	27.2 (*t*)	25.7 (*t*)
14	130.8 (*s*)	130.5 (*s*)	129.4 (*s*)
15	130.1 (*d*)	149.6 (*s*)	152.2 (*s*)
16	117.1 (*d*)	140.3 (*s*)	145.5 (*s*)
17	151.6 (*s*)	147.6 (*s*)	147.0 (*s*)
18	133.5 (*d*)	130.1 (*d*)	129.1 (*d*)
19	126.1 (*d*)	136.5 (*d*)	133.2 (*d*)
*Me*O–C(4)	56.3 (*q*)		
*Me*O–C(15)			60.6 (*q*)
*Me*O–C(16)		61.7 (*q*)	61.2 (*q*)
*Me*O–C(17)		61.4 (*q*)	61.8 (*q*)

[a]) Assignments based on HMQC and HMBC correlations; ^{13}C-NMR data of **1** and **3** at 100 MHz; of **2** at 125 MHz. [b]) In $CDCl_3$. [c]) In (D_6)acetone.

CH_2(13) with C(14), C(15), and C(18), CH_2(7) with C(5) and C(9), H–C(4) with C(3) and C(6), H–C(18) with C(1), C(13), and C(17), H–C(19) with C(2), C(3), C(6), and C(7), revealing the structure of **2** as shown. Comparison of the molecular composition and the NMR data of **2** with those of myricananin B [11] revealed that they are stereoisomers differing only in the configuration of C(8) and C(9). The two OH groups at C(8) and C(9) of **2** were spacially distant on the basis of the following evidences: *i*) significant downfield shifts of C(8) and C(9) in **2** compared to those of myricananin B, which may be due to the absence of steric compression effects of two vicinal OH groups in **2**. *ii*) In comparison of the ^{1}H,^{1}H-COSY spectra, H–C(8) exhibited no response to H–C(9) in myricananin B, but a strong coupling between H–C(8) and H–C(9) in **2**, suggesting HO–C(11) and HO–C(12) are not cofacial, in accordance with the difficulty for **2** to react with acetone, compared to myricananin B, of which an acetonide product can readily be obtained during isolation procedures. Likewise, the preferential conformation of **2** in (D_6)acetone was determined by the observed ROESY interactions, which were H–C(19) with H–C(9) and H–C(13) with H_b–C(11) (*Fig. 2*). The absolute configuration at C(8) and C(9) remain still unknown. Accordingly, the structure of **2** was assigned as (8*R**,9*S**)-16,17-dimethoxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2(19),3,5,14,16-hexaene-3,8,9,15-tetrol, with the trivial name myricananin G (**2**).

Compound **3** was obtained as colorless crystals. The molecular formula was established as $C_{22}H_{28}O_6$ by a *pseudo*-molecular ion in the HR-ESI-MS (positive-ion mode) at *m/z* 411.1785 ($[M + Na]^+$, calc. 411.1784). The similarity of the ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*, resp.) with those of **2** suggested that they are analogues. On inspection of HMBC interactions (*Fig. 1*), it was found that the main difference between **2** and **3** occurred at one Ph group. The HMBC correlations of CH_2(13) with C(15), C(16), and C(18), H–C(18) with C(2), C(15), and C(17), and three MeO groups at δ(H) 3.92, 3.97, and 3.91 with C(15), C(16), and C(17), respectively, indicated a 15,16,17-trimethoxy substitution pattern in **3**. Further, the two OH groups at C(8) and C(9) were presumed to be both β-oriented, which was supported by the observation of H–C(8) showing no response to H–C(9) in the ^{1}H,^{1}H-COSY spectrum of **3** similar to that of myricananin B, suggesting the dihedral angle of H–C(8)–C(9)–H approaching to 90°. In addition, comparison of the chemical shifts of C(8) and C(9) of myricananins A and B, **2** and **3** also revealed that two OH groups in **3** should be β-oriented. Actually, the spectroscopic data of **3** was in agreement with those of one dihydro derivative of porson which has been obtained by *Nagai et al.* through reduction of porson with $NaBH_4$ [12]. However, as a new natural product, **3** was isolated from this species for the first time. In the same manner, the preferential conformation of **3** in $CDCl_3$ was determined by ROESY experiments, which showed interactions of H–C(18) with H–C(9), H–C(11), H–C(12), and H–C(19), and of H–C(19) with H–C(8), H–C(9), and H–C(18) (*Fig. 2*). Thus, the structure of **3** was assigned as (8*S**,9*S**)-15,16,17-trimethoxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2(19),3,5,14,16-hexaene-3,8,9-triol.

The known compounds were identified as acerogenin 2-methyl ether (**4**) [13], myricanol-11-*O*-β-D-glucopyranoside (**5**) [14], myricanone-5-*O*-β-D-glucopyranoside (**6**) [14], (+)-(*S*)-myricanol 5-*O*-β-D-glucopyranoside (**7**) [14], and myricanol glucoside (**8**) [14], by comparison with literature data. All these compounds were isolated from *M. nana* for the first time.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm, *Qingdao Marine Chemical Inc.*, P. R. China), *RP-18* (40–63 μm, *Daiso Co.*, Japan), *Sephadex LH-20* (*Amersham Biosciences*, Sweden), and *MCI* gel *CHP 20P* (75–150 μm, *Mitsubishikasei*, Japan). TLC: silica gel GF_{254} (10–40 μm, *Qingdao Marine Chemical Factory*, P. R. China). Melting points: *XRC-1* micro-melting point apparatus; uncorrected. Optical rotations: *JASCO-20C* digital polarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrometer; λ_{max} in nm. IR Spectra: *Bruker Tensor 27 FT-IR* spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* spectrometer; chemical shift δ in ppm relative to Me_4Si as an internal reference, and coupling constant *J* in Hz. ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra: *DRX-500* spectrometer. MS: *VG Auto Spec-3000* mass spectrometer; in *m/z*. HR-ESI-MS: *API QSTAR Pulsar 1* spectrometer.

Plant Material. The roots of *M. nana* were collected from Songhua dam, a Kunming suburb of Yunnan Province, P. R. China, in May 2007. The species was identified by Prof. *Yumin Shui*, Kunming Institute of Botany, and a voucher specimen (CHYX0391-2) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, P. R. China.

Extraction and Isolation. The air-dried and powdered roots of *M. nana* (20 kg) were extracted three times with 80% EtOH under reflux. The extracts were concentrated and suspended in H_2O followed by successive partition with petroleum ether (PE; 3×1500 ml), AcOEt (3×1500 ml), and BuOH (3×1000 ml). The AcOEt extract (1100 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH $1:0 \rightarrow 0:1$): *Frs. 1–5. Fr. 1* (78 g) was further eluted by CC (SiO_2; $CHCl_3$/MeOH 9:1): *Frs. 1.1–1.3. Fr. 1.2* (8.3 g) was submitted to a *MCI* gel *CHP 20P* column (MeOH/H_2O $60:40 \rightarrow 100:0$): *Frs. 1.2.1–1.2.4. Fr. 1.2.1* (2.6 g) was purified by CC (*Sephadex LH-20*; MeOH): **1** (8 mg) and **4** (4 mg). *Fr. 2* (121 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 6:4): *Frs. 2.1–2.4. Fr. 2.1* (13 g) was separated by CC (*RP-18*; MeOH/H_2O $50:50 \rightarrow 90:10$): *Frs. 2.1.1–2.1.4. Fr. 2.1.4* (4.2 g) was purified by CC (*Sephadex LH-20*; MeOH/H_2O 1:1): **2** (23 mg) and **3** (3 mg). *Fr. 3* (108 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 8:2): *Frs. 3.1–3.4. Fr. 3.1* (9.2 g) was further separated by CC (*RP-18*; MeOH/H_2O $50:50 \rightarrow 100:0$): *Frs. 3.1.1–3.1.4. Fr. 3.1.3* (2.7 g) was purified by CC (*Sephadex LH-20*; MeOH): **5** (196 mg). *Fr. 4* (119 g) was separated by CC (*RP-18*; MeOH/H_2O $50:50 \rightarrow 90:10$): *Frs. 4.1–4.4. Fr. 4.4* (12.3 g) was further purified by CC (*RP-18*; MeOH/H_2O $60:40 \rightarrow 90:10$): **6** (83 mg). *Fr. 5* (98 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 3:7): *Frs. 5.1–5.4. Fr. 5.2* (3.9 g) was subjected to CC (*MCI gel CHP 20P*; MeOH/H_2O $50:50 \rightarrow 90:10$): *Frs. 5.2.1–5.2.4. Fr. 5.2.3* (1.9 g) was further purified by CC (*RP-18*; MeOH/H_2O $60:40 \rightarrow 90:10$): **7** (1.2 g) and **8** (980 mg).

Myricananin F (=*4-Methoxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2(19),3,5,14,16-hexaene-3,10,17-triol*; **1**). Amorphous white powder. M.p. 169–171°. $[\alpha]_D^{26} = +58.3$ ($c = 0.10$, MeOH). UV (MeOH): 299 (3.93), 254 (4.05), 213 (4.55). IR (KBr): 3439, 2931, 1704, 1628, 1600, 1504, 1414, 1246. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 328 (M^+). HR-ESI-MS (pos.): 351.1567 ($[M + Na]^+$, $C_{20}H_{24}NaO_4^+$; calc. 351.1572).

Myricananin G (=*(8*R**,9*S**)-16,17-Dimethoxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2(19),3,5,14,16-hexaene-3,8,9,15-tetrol*; **2**). Amorphous white powder. M.p. 223–225°. $[\alpha]_D^{26} = -3.3$ ($c = 0.10$, MeOH). UV (MeOH): 295 (3.80), 258 (4.01), 214 (4.53). IR (KBr): 3441, 2938, 1703, 1639, 1499, 1456, 1409, 1341, 1230. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 374 (M^+). HR-ESI-MS (pos.): 397.1611 ($[M + Na]^+$, $C_{21}H_{26}NaO_6^+$; calc. 397.1627).

Myricananin H (=*(8*S*,9*S*)-15,16,17-Trimethoxytricyclo[12.3.1.1^{2,6}]nonadeca-1(18),2(19),3,5,14,16-hexaene-3,8,9-triol*; **3**). Colorless crystals. M.p. 215–216°. $[\alpha]_D^{26} = +14.8$ ($c = 0.14$, MeOH). UV (MeOH): 295 (3.73), 283 (3.75), 253 (4.00), 214 (4.47). IR (KBr): 3460, 3364, 2927, 2862, 1723, 1460, 1400, 1334, 1227. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 388 (M^+). HR-ESI-MS (pos.): 411.1785 ($[M + Na]^+$, $C_{22}H_{28}NaO_6^+$; calc. 411.1784).

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