

Three New Diarylheptanoids from *Myrica nana*

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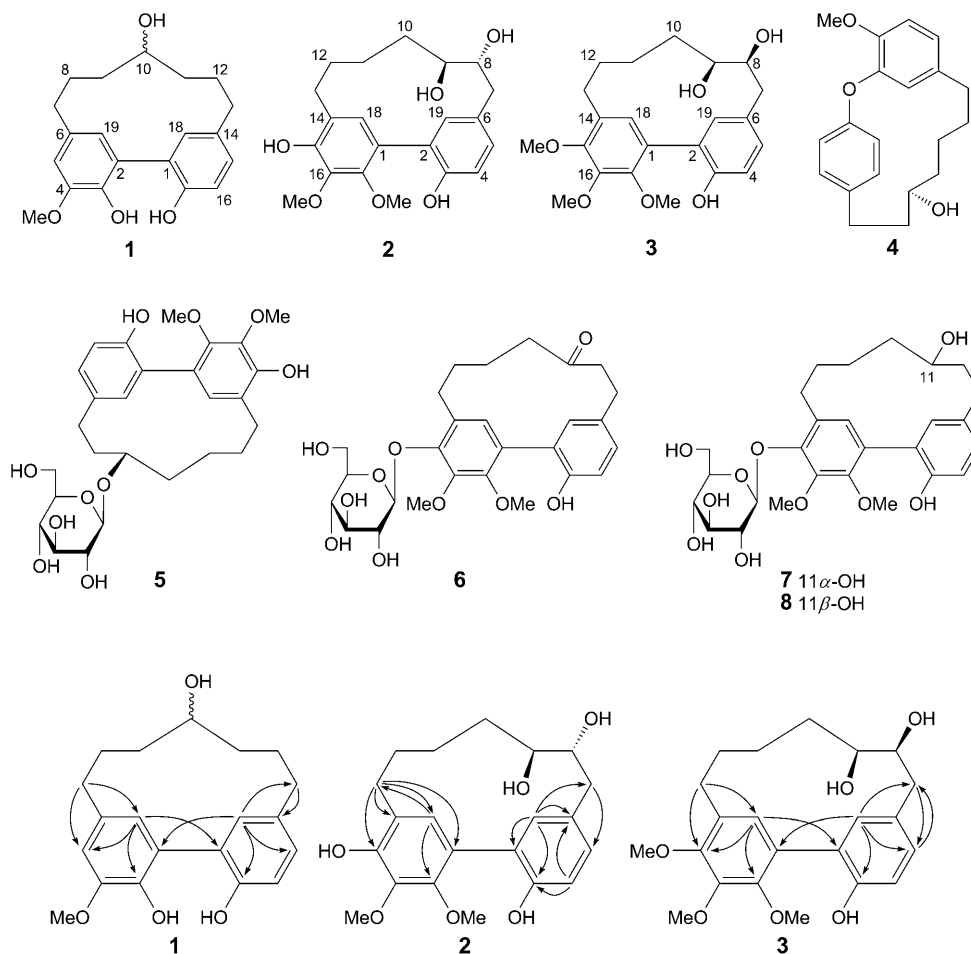
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Three new cyclic diarylheptanoids myricananins F–H (**1–3**, resp.), along with five known ones, **4–8**, were isolated from the roots of *Myrica nana*. Compound **3** has been obtained by Nagai *et al.* by reduction of porson with NaBH₄. In this work, compound **3** was isolated from natural origin for the first time. The structures of **1–8** were elucidated using spectroscopic methods.

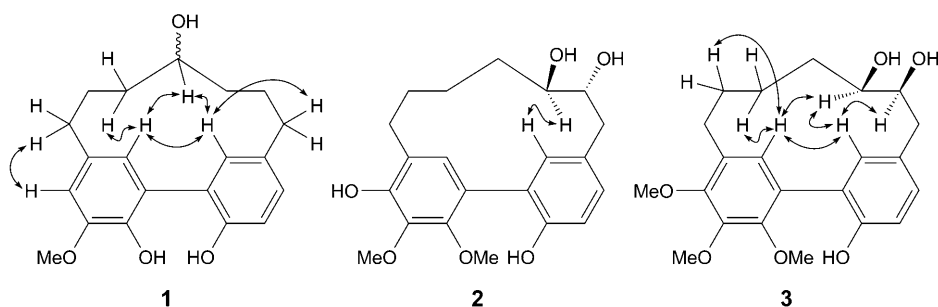
Introduction. – *Myrica nana* CHEVAL. (Myricaceae) is an evergreen shrub, mainly distributed in Yunnan and Guizhou Provinces of P. R. China [1]. Its roots are used as the folk medicine for the treatment of bleeding, diarrhea, stomach pain, and skin diseases [2]. Several chemical constituents such as 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 C₂₀H₂₄O₄ by a *pseudo*-molecular ion in the HR-ESI-MS (positive-ion mode; at *m/z* 351.1567 ([*M* + Na]⁺, calc. 351.1572)). The ¹H- and ¹³C-NMR spectroscopic data (Tables 1 and 2, resp.) of **1** exhibited the signals of one MeO group, six CH₂ 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 ¹H-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 *singlets* at δ(H) 6.67 (H–C(5)) and 6.81 (H–C(19)) in the olefinic region were also observed. The ¹H,¹H-COSY spectrum indicated the correlations from CH₂(7) to CH₂(13), and from H–C(15) to H–C(16). The HMBC spectrum showed the key correlations of CH₂(7) with C(5) and C(19), CH₂(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 $\text{H}-\text{C}(5)$ with $\text{H}_b-\text{C}(7)$, $\text{H}-\text{C}(10)$ with $\text{H}-\text{C}(18)$ and $\text{H}-\text{C}(19)$, and $\text{H}-\text{C}(19)$ with $\text{H}_a-\text{C}(9)$ and $\text{H}-\text{C}(18)$ (Fig. 2), suggesting that these H-atoms are spatially 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 $\text{C}_{21}\text{H}_{26}\text{O}_6$ by a *pseudo*-molecular-ion peak in the HR-ESI-MS (positive-ion mode; m/z 397.1611 ($[M + \text{Na}]^+$, 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. ^1H -NMR Spectroscopic Data for Compounds **1–3**^{a)}

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

^{a)} ^1H -NMR Data of **1** and **3** at 400 MHz, of **2** at 500 MHz. ^{b)} Measured in CDCl_3 . ^{c)} Measured in $(\text{D}_6)\text{acetone}$.

diarylheptanoid. The signals at $\delta(\text{H})$ 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 ^1H -NMR spectrum indicated the presence of a typical *ABX* system. ^1H , ^1H -COSY spectrum implied two spin systems, which were $\text{CH}_2(7)$ to $\text{CH}_2(13)$ and $\text{H}-\text{C}(4)$ to $\text{H}-\text{C}(5)$. 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)
MeO–C(4)	56.3 (q)		
MeO–C(15)			60.6 (q)
MeO–C(16)		61.7 (q)	61.2 (q)
MeO–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 $(\text{D}_6)\text{acetone}$.

$\text{CH}_2(13)$ with C(14), C(15), and C(18), $\text{CH}_2(7)$ 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 ^1H , ^1H -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 acetone product can readily be obtained during isolation procedures. Likewise, the preferential conformation of **2** in $(\text{D}_6)\text{acetone}$ 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 1H - 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 $\delta(H)$ 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 $^1H, ^1H$ -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}]nona-deca-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.

This work was financially supported by the following grants: A ‘Talent Scholarship for the Youth of Yunnan’ (No. 2007PY01-48), ‘Xi-Bu-Zhi-Guang’ Project from the Chinese Academy of Sciences, P. R. China.

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 , Mitsubishi Kasei, Japan). TLC: silica gel GF₂₅₄ (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. $^1H, ^1H$ -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 H₂O 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 (SiO₂; CHCl₃/MeOH 1:0 → 0:1): *Frs. 1* – 5. *Fr. 1* (78 g) was further eluted by CC (SiO₂; CHCl₃/MeOH 9:1): *Frs. 1.1* – 1.3. *Fr. 1.2* (8.3 g) was submitted to a MCI gel CHP 20P column (MeOH/H₂O 60:40 → 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 (SiO₂; CHCl₃/MeOH 6:4): *Frs. 2.1* – 2.4. *Fr. 2.1* (13 g) was separated by CC (RP-18; MeOH/H₂O 50:50 → 90:10): *Frs. 2.1.1* – 2.1.4. *Fr. 2.1.4* (4.2 g) was purified by CC (Sephadex LH-20; MeOH/H₂O 1:1): **2** (23 mg) and **3** (3 mg). *Fr. 3* (108 g) was subjected to CC (SiO₂; CHCl₃/MeOH 8:2): *Frs. 3.1* – 3.4. *Fr. 3.1* (9.2 g) was further separated by CC (RP-18; MeOH/H₂O 50:50 → 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/H₂O 50:50 → 90:10): *Frs. 4.1* – 4.4. *Fr. 4.4* (12.3 g) was further purified by CC (RP-18; MeOH/H₂O 60:40 → 90:10): **6** (83 mg). *Fr. 5* (98 g) was separated by CC (SiO₂; CHCl₃/MeOH 3:7): *Frs. 5.1* – 5.4. *Fr. 5.2* (3.9 g) was subjected to CC (MCI gel CHP 20P; MeOH/H₂O 50:50 → 90:10): *Frs. 5.2.1* – 5.2.4. *Fr. 5.2.3* (1.9 g) was further purified by CC (RP-18; MeOH/H₂O 60:40 → 90:10): **7** (1.2 g) and **8** (980 mg).

Myricanenin 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°. $[\alpha]_D^{25} = +58.3$ ($c = 0.10$, MeOH). UV (MeOH): 299 (3.93), 254 (4.05), 213 (4.55). IR (KBr): 3439, 2931, 1704, 1628, 1600, 1504, 1414, 1246. ¹H- and ¹³C-NMR: *Tables 1* and 2. ESI-MS (pos.): 328 (M^+). HR-ESI-MS (pos.): 351.1567 ($[M + Na]^+$, C₂₀H₂₄NaO₄⁺; calc. 351.1572).

Myricanenin G (= (8R*,9S*)-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°. $[\alpha]_D^{25} = -3.3$ ($c = 0.10$, MeOH). UV (MeOH): 295 (3.80), 258 (4.01), 214 (4.53). IR (KBr): 3441, 2938, 1703, 1639, 1499, 1456, 1409, 1341, 1230. ¹H- and ¹³C-NMR: *Tables 1* and 2. ESI-MS (pos.): 374 (M^+). HR-ESI-MS (pos.): 397.1611 ($[M + Na]^+$, C₂₁H₂₆NaO₆⁺; calc. 397.1627).

Myricanenin H (= (8S,9S)-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°. $[\alpha]_D^{25} = +14.8$ ($c = 0.14$, 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. ¹H- and ¹³C-NMR: *Tables 1* and 2. ESI-MS (pos.): 388 (M^+). HR-ESI-MS (pos.): 411.1785 ($[M + Na]^+$, C₂₂H₂₈NaO₆⁺; calc. 411.1784).

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Received January 26, 2009