Lignans from the Bark of *Zanthoxylum planispinum*

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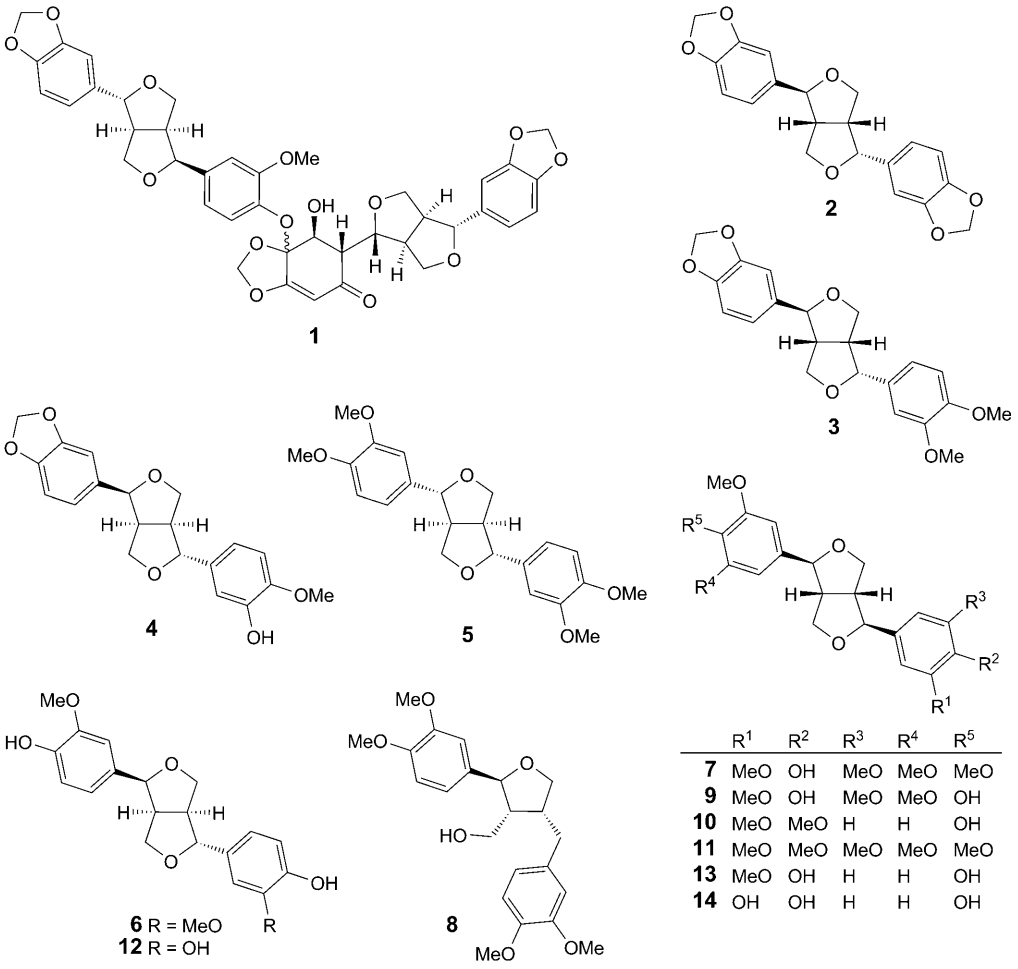
A new dimeric lignan, named biplanispine A (**1**) which represents the first example of a dimeric 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane skeleton from a *Rutaceae* plant, together with 13 known lignans **2**–**14**, was isolated from the bark of *Zanthoxylum planispinum*. Their structures were elucidated by spectroscopic methods, especially 2D-NMR techniques. All isolated compounds were evaluated for their antibacterial activities by the disc diffusion method. Compounds **7** and **9** showed interesting antibacterial activities at 200 μg/disc; they were as effective as kanamycin sulfate at 10 μg/disc against *Staphylococcus aureus* and *Bacillus subtilis*.

Introduction. – The genus *Zanthoxylum* belongs to the Rutaceae family, which comprise 250 species distributed in tropical and subtropical zones of Asia, America, Africa, and Oceania, and there are 45 species and 13 mutations in China [1]. Chemical constituents of *Zanthoxylum* have been studied extensively. Previous phytochemical investigations on this species revealed that benzophenanthridine alkaloids [2], coumarins [3], amides [4], and lignans [5] are largely represented in this genus. Lignan constituents have been reported to possess anticancer, antimicrobial, antioxidant, anti-inflammatory, and immunosuppressive activities [6]. *Zanthoxylum planispinum* is widely used as a folk medicine for treating colds, preventing toothache, and expelling roundworms [7]. Chemical studies of the bark of *Z. planispinum* have not been conducted previously. In our search for bioactive compounds from the crude extract of ethnomedicinal plants, we found that an AcOEt-soluble partition of the EtOH extract of the bark of *Z. planispinum* exhibited potent antibacterial activities against some microbes. This prompted us to perform a detailed bioassay-guided investigation of this plant. As a result, a new dimeric lignan, named biplanispine A (**1**), which is the first example of dimeric 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane skeleton from *Rutaceae* plant, together with 13 known lignans **2**–**14**, were isolated from the bark of *Z. planispinum*. Their structures were elucidated by spectroscopic methods, especially 2D-NMR techniques. This article describes the structural investigation of these natural products and their antibacterial activities.

Results and Discussion. – Compound **1**, which was obtained as colorless amorphous powder, exhibited an $[M + Na]^+$ ion peak at m/z 765.2140 in the HR-ESI-MS (positive-ion mode), corresponding to the molecular formular $C_{40}H_{38}O_{14}$. The ^{1}H- and ^{13}C-NMR



	R^1	R^2	R^3	R^4	R^5
7	MeO	OH	MeO	MeO	MeO
9	MeO	OH	MeO	MeO	OH
10	MeO	MeO	H	H	OH
11	MeO	MeO	MeO	MeO	MeO
13	MeO	OH	H	H	OH
14	OH	OH	H	H	OH

data (*Tables 1* and *2*) suggested that **1** possesses two 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane units. ^{1}H,^{1}H-COSY, HMBC (*Tables 1* and *2*) and ROESY experiments, as well as comparison with known compounds established the structure of **1** as shown in the *Figure*, and was named biplanispine A.

The ^{1}H- and ^{13}C-NMR data revealed that part **A** of **1** resembled those of the known compound **3** [8]. The ^{1}H-NMR spectrum showed two CH groups at δ(H) 2.47–2.49 (*m*, 1 H) and at 3.38–3.42 (*m*, 1 H), two benzylic O-bearing CH moieties at δ(H) 4.34 (*d*, J = 7.2, 1 H) and 4.78 (*d*, J = 6.0, 1 H), two O-bearing CH_2 groups at δ(H) 4.05 (*d*, J = 9.0, 1 H) and 3.68–3.76 (*m*, 1 H), 2.88 (*dd*, J = 8.4, 7.8, 1 H), and 2.99 (*dd*, J = 9.0, 7.8, 1 H), six aromatic H-atoms as two *ABX* systems at δ(H) 6.82–7.18 indicating the presence of two 1,3,4-trisubstituted benzene rings, in addition to one MeO group at δ(H) 3.63 (*s*, 3 H), and of one OCH_2O group at δ(H) 5.96 (*s*, 2 H). Accordingly, part **A** of **1** was assigned to be a 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane bearing one MeO

Table 1. *^{1}H- and ^{13}C-NMR, and HMBC Data* ((D_6)DMSO) *of Part* **A** *of Compound* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC (H → C)
H–C(1)	2.47–2.49 (*m*)	54.0	C(9)
H–C(2)	4.34 (*d*, *J* = 7.2)	86.7	C(1), C(8), C(9), C(10), C(14)
CH_2(4)	2.88 (*dd*, *J* = 8.4, 7.8), 2.99 (*dd*, *J* = 9.0, 7.8)	68.9	
H–C(5)	3.38–3.42 (*m*)	49.2	
H–C(6)	4.78 (*d*, *J* = 6.0)	81.1	C(4), C(5), C(15), C(16), C(20)
CH_2(8)	3.68–3.76 (*m*), 4.05 (*d*, *J* = 9.0)	70.3	C(2), C(5), C(6)
C(9)		135.5	
H–C(10)	6.89 (br. *s*)	106.5	C(2), C(11), C(12), C(14)
C(11)		147.4	
C(12)		146.6	
H–C(13)	6.82–6.86 (*m*)	108.0	
H–C(14)	6.82–6.86 (*m*)	119.4	
C(15)		136.6	
H–C(16)	6.94 (br. *s*)	109.9	C(6), C(17), C(18), C(20)
C(17)		152.0	
C(18)		140.8	
H–C(19)	6.82–6.86 (*m*)	117.5	
H–C(20)	7.18 (*d*, *J* = 8.4)	124.4	C(15), C(17), C(18)
–OCH_2O–	5.96 (*s*)	100.9	C(11), C(12)
MeO	3.63 (*s*)	55.3	C(17)

Table 2. *^{1}H- and ^{13}C-NMR, and HMBC Data* ((D_6)DMSO) *of Part* **B** *of Compound* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC (H → C)
H–C(1′)	3.74–3.76 (*m*)	48.4	C(8′)
H–C(2′)	3.92–3.96 (*m*)	83.8	
CH_2(4′)	3.48 (*dd*, *J* = 8.4, 7.8), 3.66–3.74 (*m*)	69.1	C(1′), C(2′)
H–C(5′)	3.26–3.32 (*m*)	49.4	
H–C(6′)	4.73 (*d*, *J* = 5.4)	80.9	C(4′), C(5′), C(15′), C(16′), C(20′)
CH_2(8′)	3.68–3.76 (*m*), 3.85 (*d*, *J* = 8.4)	70.8	C(2′), C(5′), C(6′)
H–C(9′)	2.92 (*d*, *J* = 10.2)	50.6	C(2′), C(10′), C(14′)
H–C(10′)	3.96–4.00 (*m*)	72.1	C(14′)
C(11′)		105.3	
C(12′)		167.0	
H–C(13′)	5.41 (*s*)	102.0	C(9′), C(11′), C(12′)
C(14′)		196.4	
C(15′)		132.8	
H–C(16′)	6.82–6.86 (*m*)	106.2	
C(17′)		147.0	
C(18′)		145.9	
H–C(19′)	6.82–6.86 (*m*)	107.9	
H–C(20′)	6.77 (*d*, *J* = 7.8)	118.6	C(16′), C(18′)
–OCH_2O–	5.96 (*s*)	100.8	C(17′), C(18′)
–OCH_2O–	5.25 (*s*), 5.59 (*s*)	100.3	C(11′), C(12′)
HO–C(10′)	5.84 (*d*, *J* = 7.2)		C(9′), C(10′)

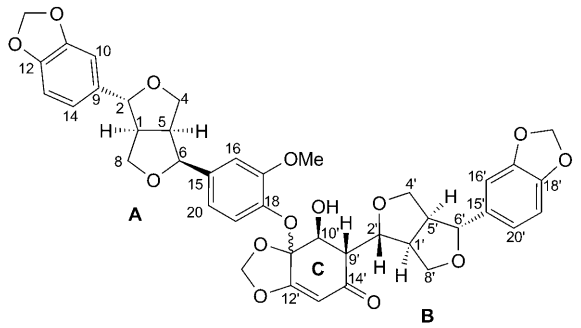


Figure. *The structure and arbitrary atom numbering of compound* **1**

group, one O-functional group and one OCH_2O group. This assumption was further supported by the ^{13}C-NMR data including two CH groups (δ(C) 54.0 and 49.2), two benzylic O-bearing CH groups (δ(C) 86.7 and 81.1), two O-bearing CH_2 groups (δ(C) 70.3 and 68.9), six aromatic C-atoms and six aromatic CH groups. The ^{1}H- and ^{13}C-NMR data of part **A** of **1** were similar to those of **3** [8], suggesting one MeO group of **3** was replaced by an O-functional group in **1**, which was supported by an MS fragment at *m*/*z* 356. In the HMBC spectrum, the correlations of δ(H) 3.63 (*s*, MeO)/δ(C) 152.0 (C(17)) indicated that a MeO group was located at C(17). Furthermore, the ROESY spectrum allowed us to confirm the position of the MeO group, showing that the MeO signal correlates with H–C(16). The relative configuration of part **A** of **1** was determined on the basis of a ROESY experiment. The ROESY correlations between H_β–C(2)/H_β–C(8) and H_β–C(4) indicated that these H-atoms were all on the same face of the molecule and had β orientation, whereas those observed with H_α–C(6)/H_α–C(8) and H_α–C(5), and H_α–C(1)/H_α–C(5) established that these H-atoms were on the other side of the molecule and had α orientation. The linkage between C(1) and C(5) was confirmed to be *cis*, which is typical of naturally occuring bis-tetrahydrofuran lignans [9]. From the ROESY data, part **A** of **1** was concluded to be an unsymmetrically substituted bis-tetrahydrofuran lignan, possessing an equatorial aromatic ring at C(2) and an axial aromatic ring at C(6).

Except for the ^{1}H- and ^{13}C-NMR signals of part **A** mentioned above, signals of three H-atoms of an *ABX* system at δ(H) 6.77–6.86 (*m*), an OCH_2O at δ(H) 5.96 (*s*, 2 H), together with three aromatic C-atoms (δ(C) 132.8, 147.0, 145.9) and three aromatic CH groups (δ(C) 106.2, 107.9, 118.6) were attributed to a 3,4-methylenedioxyphenyl group from the HMBC spectrum. Signals of two O-bearing CH groups (δ(H)/δ(C) 3.92–3.96 (*m*, 1 H)/83.8 (C(2′)) and 4.73 (*d*, *J* = 5.4, 1 H)/80.9 (C(6′))), two O-bearing CH_2 groups (δ(H)/δ(C) 3.48 (*dd*, *J* = 8.4, 7.8, 1 H), 3.66–3.74 (*m*, 1 H)/69.1 (C(4′)) and 3.68–3.76 (*m*, 1 H), 3.85 (*d*, *J* = 8.4, 1 H)/70.8 (C(8′))), and two CH groups (δ(H)/δ(C) 3.74–3.76 (*m*, 1 H)/48.4 (C(1′)) and 3.26–3.32 (*m*, 1 H)/49.4 (C(5′))) were also apparent in the ^{1}H- and ^{13}C-NMR spectra. By analysis of the ^{1}H,^{1}H-COSY and HSQC spectra, the correlation of H–C(2′) with H–C(1′), of H–C(1′) with H–C(5′) and CH_2(8′), and of H–C(5′) with H–C(6′) and CH_2(4′) gave the partial structure –OCH–CH(CH_2O)–CH(CH_2O)–CHO– which was consistent with the HMBC spectrum. The HMBC between H–C(6′) and C(4′) and C(5′), between CH_2(4′) and

C(1′) and C(2′), and between CH_2(8′) and C(2′), C(5′), and C(6′) were used to establish the presence of the 3,7-dioxabicyclo[3.3.0]octane skeleton in part **B** of **1**. In the HMBC spectrum, the correlations of H–C(6′) at δ(H) 4.73 (*d*, $J = 5.4$, 1 H) to δ(C) 132.8 (C(15′)), 106.2 (C(16′)), and 118.6 (C(20′)) suggested that the 3,4-methylenedioxyphenyl group should be located at C(6′) position. The remaining structure parts were identified by ^{1}H- and ^{13}C-NMR (DEPT) as two CH (one O-bearing), one OCH_2O, one α,β-unsaturated keto group at δ(C) 196.4, one doubly O-bearing cyclic ketal C-atom at δ(C) 105.3, one C=C bond with signals at δ(C) 102.0 (CH) and 167.0 (C), and one OH group.

The aforementioned groups accounted for 21 out of 22 degrees of unsaturation in compound **1**, indicating the presence of an additional ring. From the ^{1}H,^{1}H-COSY spectrum, the correlation of HO–C(10′) at δ(H) 5.84 (*d*, $J = 7.2$, 1 H) with H–C(10′) at δ(H) 3.96–4.00 (*m*, 1 H) and H–C(10′) with H–C(9′) at δ(H) 2.92 (*d*, $J = 10.2$, 1 H) led to the partial structure C(10′)–C(9′). The HMBC cross-peaks between the OCH_2O group at δ(H) 5.25 (*s*, 1 H) and 5.59 (*s*, 1 H) and δ(C) 105.3 (C(11′)) and δ(C) 167.0 (C(12′)) indicated that the OCH_2O group was attached to C(11′) and C(12′). The correlation of H–C(13′) at δ(H) 5.41 (*s*, 1 H) with C(11′) and C(12′) established the partial structure C(11′)–C(14′). The HMBC correlations of H–C(13′) with C(9′) and of H–C(9′) with C(10′) and C(14′) indicated that C(9′) and C(10′) were connected with C(14′) and C(11′), respectively, and established the remaining structure as depicted as ring **C** (see *Fig.*). This ring is located at C(2′) based on the HMBC correlation of H–C(9′) with C(2′).

The relative configuration of part **B** of **1** was established on the basis of ^{1}H-NMR data and a ROESY experiment. The chemical shift observed for H–C(6′) at δ(H) 4.73 (*d*, $J = 5.4$, 1 H) was in accordance with that reported where the 3,4-methylenedioxyphenyl group was equatorial [10], and H–C(6′) and H–C(5′) was *trans*-oriented, which was confirmed by the ROESY correlation between H–C(6′) and H_β–C(8′). The ROESY correlation between H–C(1′) and H–C(9′) indicated that the substituent at C(2′) is *syn* to H–C(1′). The large coupling constant $J = 10.2$ Hz between H–C(9′) and H–C(10′) suggested that H–C(9′) and H–C(10′) are arranged *trans* to each other [11]. The OCH_2O group at δ(H) 5.25 (*s*, 1 H) and 5.59 (*s*, 1 H) showed no ROESY correlations with other H-atoms. Thus, the relative configuration at C(11′) could not be determined from the ROESY spectrum. From these results, the partial structure **B** of **1** was confirmed. Since C(18) of part **A** and C(11′) of part **B** were O-bearing quaternary C-atoms, compound **1** is suggested to be a dimeric lignan linked through the dehydrogenation between HO–C(18) and a hemiacetal system at C(11′) to form an ether linkage. Therefore, biplanispine A was deduced to be as shown for **1**. However, the absolute configuration of **1** was not determined.

Naturally occuring dilignans from *Rutaceae* plants are rare. There are some reports on dilignans such as arctignan F isolated from *Arctium fructus* and *Wikstroemai indica* [12]. However, biplanispine A (**1**) is the first example of a dilignan with two 3,7-dioxabicyclo[3.3.0]octane system from *Rutaceae* plant.

The 13 known compounds were identified as asarinin (**2**) [13], fargesin (**3**) [8], horsfieldin (**4**) [14], eudesmin (**5**) [15], epipinoresinol (**6**) [8], de-4′-*O*-methylyangambin (**7**) [16], lariciresinol dimethyl ether (**8**) [17], syringaresinol (**9**) [8], pinoresinol monomethyl ether (**10**) [8], yangambin (**11**) [18], 3′-*O*-demethylepipinoresinol (**12**)

[19], pinoresinol (**13**) [8], and (1*R*,2*S*,5*R*,6*S*)-6-(4-hydroxy-3-methoxyphenyl)-2-(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane (**14**) [20] by comparison of their spectroscopic data with those reported in the literature. Notably, all reported lignans were isolated from *Z. planispinum* for the first time.

All isolated compounds were evaluated for their antibacterial activities by the disc diffusion method [21]. The results are shown in *Table 3*. At 200 μg/disc **9** showed 13 and 16 mm diameter of the inhibition zone against *Staphylococcus aureus* and *Bacillus subtilis*, respectively. The strain *B. subtilis* showed 13-mm inhibition diameter, and the strain *S. aureus* showed 10 mm inhibition diameter in the presence of **7**. The other compounds showed no activities against the two bacteria strains at the same concentration.

Table 3. *Antibacterial Activities of Lignans Isolated from* Z. planispinum[a])

Compound	Bacteria	
	S. aureus	*B. subtilis*
1	6[b])	6
2[c])	6	6
3	6	6
4	6	6
5	6	6
6	6	6
7	10	13
8	6	6
9	13	16
10	6	6
11	6	6
12	6	6
13	6	6
14	6	6
kanamycin sulfate	19	19

[a]) Values are means of three determinations. [b]) Diameter of the inhibition zone in mm, including the diameter of the filter paper disc (6 mm). [c]) 200 μg Compound/disc, or 10 μg kanamycin sulfate/disc, respectively.

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Experimental Part

General. TLC: Precoated silica gel GF_{254} plates (*Qingdao Haiyang Chemical Co., Ltd.*, P. R. China). Column Chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China) and C_{18} reversed-phase silica gel (*YMC CO., LTD.*, Japan). HPLC: *Ultimate 3000* HPLC system; *Ultimate 3000* pump; *Ultimate 3000* Variable Wavelength; column *Waters* $5C_{18}$*-MS-II* (10 × 250 mm). Optical rotation: *Perkin-Elmer 341* polarimeter. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-300* and *600* instrument; δ in ppm rel. to Me_4Si as internal standard (= 0 ppm), *J* in Hz. EI-MS and HR-EI-MS: *Finnigan MAT-95* mass spectrometer (70 eV); in *m*/*z* (rel. %). ESI-MS and HR-ESI-MS: *Finnigan* LCQ-*Deca* and *Waters/Micromass Q-Tof-Ultima* mass spectrometers, resp., in *m*/*z* (rel. int).

Plant Material. The barks of *Z. planispinum* were collected from Badong County, Hubei Province, P. R. China and identified by Prof. *Dinrong Wan*, College of Pharmacy, South Central University for Nationalities. A voucher specimen was deposited with the Herbarium of College of Pharmacy, South Central University for Nationalities.

Extraction and Isolation. The milled, air-dried barks of *Zanthoxylum planispinum* (17 kg) were powdered and then extracted three times with MeOH at r.t., and the MeOH extract (1.6 kg) was suspended in 90% H_2O/MeOH and then successively extracted with petroleum ether (PE), AcOEt, and BuOH. The AcOEt extract (47 g) was subjected to CC (SiO_2; cyclohexane/Me_2CO 95:5, 9:1, 8:2, 7:3, 1:1, and 0:1): *Frs. 1–8*. Compounds **2** (100 mg) and **3** (2.8 g) were crystallized from *Fr. 3* and *Fr. 5*, resp. *Fr. 6* (8.2 g) was subjected to CC (SiO_2, cyclohexane/AcOEt 9:1 → 0:1): *Frs. 6.1–6.6*. Compound **4** (30 mg) was crystallized from *Fr. 6.2*. *Fr. 6.3* (3.9 g) was subjected to CC (*ODS*; H_2O/MeOH 7:3 → 0:1): *Frs. 6.3.1 – 6.3.7*. *Fr. 6.3.2* (1.3 g) was subjected to CC (SiO_2, cyclohexane/AcOEt 8:2 → 0:1): *Frs. 6.3.2.1 – 6.3.2.4*. *Fr. 6.3.2.3* (73.9 mg) was further purified by CC (SiO_2; $CHCl_3$/MeOH 1:0 → 0:1): **5** (30 mg). *Fr. 6.4* (2.2 g) was subjected to CC (SiO_2; $CHCl_3$/AcOEt 1:0 → 0:1): *Frs. 6.4.1 – 6.4.8*. *Fr. 6.4.4* (187.4 mg) was purified by CC (SiO_2; cyclohexane/AcOEt 9:1 → 0:1) and followed by CC (SiO_2; PE/Me_2CO 8:2 → 0:1): **6** (16.4 mg). *Fr. 6.5* (1.3 g) was subjected to CC (*ODS*; H_2O/MeOH 7:3 → 0:1): *Frs. 6.5.1 – 6.5.8*. *Fr. 6.5.2* (138 mg) was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0 → 0:1): **7** (16.8 mg) and **8** (25 mg). Compound **1** (20 mg) was crystallized from *Fr. 6.5.6*. *Fr. 7* (2.1 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0 → 0:1): **9** (5.5 mg).

The remaining AcOEt extract (307 g) was subjected to CC (SiO_2; PE/Me_2CO 9:1, 8:2, 7:3, 1:1, and 0:1): *Frs. 1–15*. *Fr. 9* (52.3 g) was subjected to CC (SiO_2; cyclohexane/AcOEt 9:1 → 0:1): *Frs. 9.1 – 9.13*. *Fr. 9.8* (8.9 g) was subjected to CC (*ODS*; H_2O/MeOH 7:3 → 0:1) and then further purified by semi-prep. HPLC (MeOH/H_2O 60:40, 3 ml/min): **10** (44 mg) at t_R 15.1 min, **11** (10 mg) at 23.2 min. *Fr. 9.10* (8.2 g) was subjected to CC (*ODS*; H_2O/MeOH 7:3 → 0:1): *Frs. 9.10.1 – 9.10.12*. *Fr. 9.10.5* was further purified by semi-prep. HPLC (MeOH/H_2O 60:40, 3 ml/min; t_R 32.7 min): **12** (20 mg). *Fr. 9.10.7* was further purified by CC (SiO_2; cyclohexane/AcOEt 9:1 → 0:1): **13** (5 mg). *Fr. 10* (90 g) was subjected to CC (SiO_2; $CHCl_3$/Me_2CO 98:2 → 0:1): *Frs. 10.1 – 10.7*. *Fr. 10.4* (3.9 g) was subjected to CC (*ODS*; H_2O/MeOH 7:3 → 0:1): *Frs. 10.4.1 – 10.4.7*. *Fr. 10.4.3* (135 mg) was purified by CC (SiO_2; cyclohexane/AcOEt 9:1 → 0:1): **14** (17.6 mg).

Biplanispine A (=*(6*R*,7*S*)-6-[(3a*S*,4*R*,6a*S*)-4-(1,3-Benzodioxol-5-yl)tetrahydro-1*H,3*H-furo[3,4-c]furan-1-yl]-7a-[4-[(1*S*,3a*S*,4*R*,6a*S*)-4-(1,3-benzodioxol-5-yl)tetrahydro-1*H,3*H-furo[3,4-c]furan-1-yl]-2-methoxyphenoxy}-7-hydroxy-7,7a-dihydro-1,3-benzodioxol-5(6*H)-one*; **1**). Colorless amorphous powder. $[\alpha]_D = -9.3$ ($c = 0.1$, DMSO). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. EI-MS: 356 (76), 151 (60), 149 (100), 135 (68), 131 (36). HR-ESI-MS: 765.2140 ($[M+Na]^+$, $C_{40}H_{38}NaO_{14}^+$; calc. 765.2159).

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