Schisanhenones A and B, Two Metabolites of Schisanhenol in Rats

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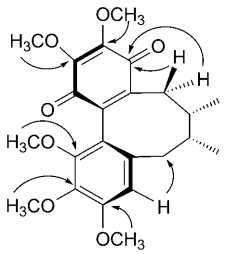
Schisanhenones A (**2**) and B (**3**), two new metabolites of schisanhenol (**1**), were isolated from the feces of rats administered orally with **1**. Their structures and configurations were elucidated by spectroscopic methods including 2D-NMR techniques.

Introduction. – Schisanhenol (**1**) is one of the natural lignans isolated from the fruits of *Schisandra rubriflora* RHED. et WILS (Schisandraceae), which is commonly used as tonic, sedative, and astringent agent in China [1]. Pharmacological studies have revealed that **1** exerts an antioxidant effect on some chemical-induced cell injuries [2–4] and an inhibitory effect on platelet aggregation induced by ADP and PAF *in vitro* [5]. Schisanhenol is a valuable natural product worthy to be investigated as a potential drug for treating ischemic cerebral apoplexy [6]. To clarify the metabolic features of this bioactive natural product, we administered rats with schisanhenol (**1**) orally and collected the feces, from which the two new oxidative metabolites **2** and **3** with changed biphenyl configuration were obtained and characterized. The structure determination of these two new metabolites is reported herein.

Results and Discussion. – Oral administration of schisanhenol to rats yielded the two metabolites schisanhenones A (**2**) and B (**3**), which are described for the first time.

Compound **2**, obtained as a reddish amorphous solid, has the molecular formula $C_{23}H_{28}O_7$ which was deduced from its HR-ESI-MS (*m*/*z* 439.1740 ($[M+Na]^+$)). The UV spectrum of **2** showed maximum absorptions at 215 and 276 nm which, along with the ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) and their comparison with those of **1**, indicated that **2** was a dibenzocyclooctene-type lignan. The IR, HMBC (*Fig. 1*), CD,



2

Fig. 1. *Key HMBC observed for* **2**

Table 1. *^{1}H-NMR Data* ($CDCl_3$, 400 MHz; 27°) *of* **2**, **2a**, **3**, *and* **3a**. δ in ppm, J in Hz.

	2	**2a**	**3**	**3a**
H_α–C(5)	1.87 (*d*, *J* = 13.9)	2.35 (*dd*, *J* = 13.3, 10.2)	1.88 (*d*, *J* = 13.9)	2.33 (*dd*, *J* = 13.3, 9.4)
H_β–C(5)	3.17 (*dd*, *J* = 13.9, 8.8)	2.20 (*d*, *J* = 13.3)	3.18 (*dd*, *J* = 13.9, 8.8)	2.19 (*d*, *J* = 13.3)
H–C(6)	1.92–1.95 (*m*)	1.74–1.76 (*m*)	1.92–1.94 (*m*)	1.78–1.80 (*m*)
H–C(7)	1.70–1.73 (*m*)	1.96–1.99 (*m*)	1.71–1.74 (*m*)	1.94–1.96 (*m*)
H_α–C(8)	2.20 (*d*, *J* = 13.5)	2.71 (*dd*, *J* = 7.1, 13.9)	2.19 (*d*, *J* = 13.2)	2.68 (*dd*, *J* = 7.4, 13.3)
H_β–C(8)	2.35 (*dd*, *J* = 13.5, 10.3)	2.47 (*d*, *J* = 13.9)	2.32 (*dd*, *J* = 13.2, 9.9)	2.42 (*d*, *J* = 13.3)
H–C(9)	6.52 (*s*)	6.53 (*s*)	6.62 (*s*)	6.63 (*s*)
Me–C(6)	0.75 (*d*, *J* = 7.0)	1.04 (*d*, *J* = 7.0)	0.75 (*d*, *J* = 7.0)	1.00 (*d*, *J* = 7.0)
Me–C(7)	1.00 (*d*, *J* = 7.0)	0.72 (*d*, *J* = 7.0)	0.98 (*d*, *J* = 7.0)	0.72 (*d*, *J* = 7.0)
MeO–C(2)	4.04 (*s*)	4.03 (*s*)	4.04 (*s*)	4.04 (*s*)
MeO–C(3)	4.04 (*s*)	4.03 (*s*)	4.04 (*s*)	4.04 (*s*)
MeO–C(10)	3.89 (*s*)	3.88 (*s*)	–	–
MeO–C(11)	3.84 (*s*)	3.85 (*s*)	3.87 (*s*)	3.88 (*s*)
MeO–C(12)	3.77 (*s*)	3.79 (*s*)	3.73 (*s*)	3.76 (*s*)
OH–C(10)	–	–	5.80 (*s*)	5.79 (*s*)

Table 2. *^{13}C-NMR Data* ($CDCl_3$, 100 MHz; 27°) *of* **2**, **2a**, **3**, *and* **3a**. δ in ppm, J in Hz.

	2	**2a**	**3**	**3a**
C(1)	183.2 (*s*)	183.2 (*s*)	182.7 (*s*)	180.6 (*s*)
C(2)	144.5 (*s*)	144.3 (*s*)	144.1 (*s*)	144.6 (*s*)
C(3)	144.5 (*s*)	144.3 (*s*)	144.1 (*s*)	144.6 (*s*)
C(4)	184.3 (*s*)	184.2 (*s*)	184.0 (*s*)	183.8 (*s*)
C(4a)	141.8 (*s*)	139.4 (*s*)	141.8 (*s*)	141.8 (*s*)
C(5)	30.6 (*t*)	27.4 (*t*)	30.4 (*t*)	27.1 (*t*)
C(6)	32.4 (*d*)	36.6 (*d*)	32.1 (*d*)	36.4 (*d*)
C(7)	39.5 (*d*)	33.1 (*d*)	39.3 (*d*)	32.9 (*d*)
C(8)	36.2 (*t*)	39.7 (*t*)	35.5 (*t*)	39.1 (*t*)
C(8a)	138.7 (*s*)	134.2 (*s*)	139.3 (*s*)	134.6 (*s*)
C(9)	107.6 (*d*)	110.9 (*d*)	110.4 (*d*)	113.5 (*d*)
C(10)	154.5 (*s*)	153.4 (*s*)	150.2 (*s*)	149.2 (*s*)
C(11)	139.3 (*s*)	139.4 (*s*)	136.8 (*s*)	136.8 (*s*)
C(12)	150.6 (*s*)	150.7 (*s*)	149.3 (*s*)	149.3 (*s*)
C(12a)	118.5 (*s*)	118.6 (*s*)	117.6 (*s*)	117.7 (*s*)
C(12b)	140.7 (*s*)	138.7 (*s*)	140.2 (*s*)	140.2 (*s*)
Me–C(6)	12.6 (*q*)	21.1 (*q*)	12.3 (*q*)	20.7 (*q*)
Me–C(7)	20.8 (*q*)	11.6 (*q*)	20.4 (*q*)	11.1 (*q*)
*Me*O–C(2)	61.1 (*q*)	61.1 (*q*)	60.8 (*q*)	60.8 (*q*)
*Me*O–C(3)	61.1 (*q*)	61.1 (*q*)	60.8 (*q*)	60.8 (*q*)
*Me*O–C(10)	55.9 (*q*)	56.0 (*q*)	–	–
*Me*O–C(11)	60.7 (*q*)	60.7 (*q*)	60.2 (*q*)	60.3 (*q*)
*Me*O–C(12)	60.9 (*q*)	60.9 (*q*)	59.9 (*q*)	59.9 (*q*)

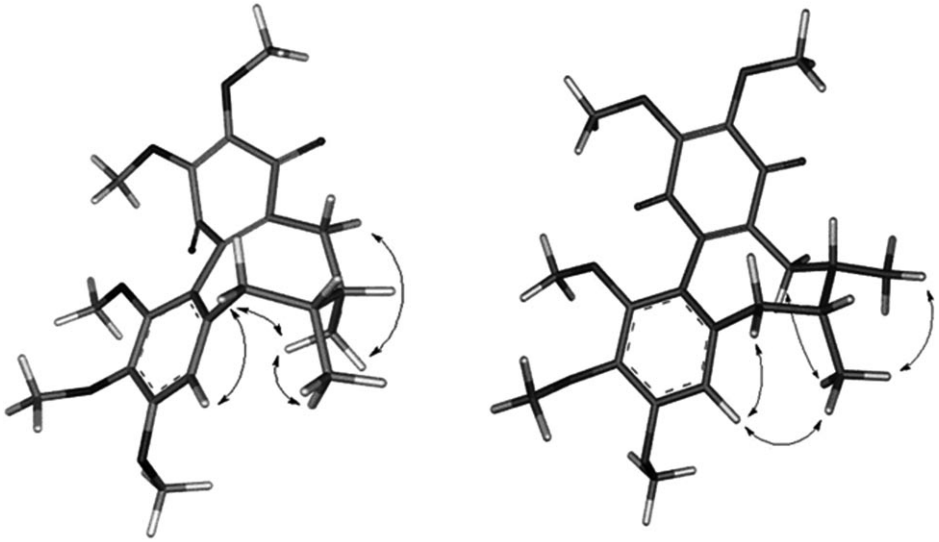


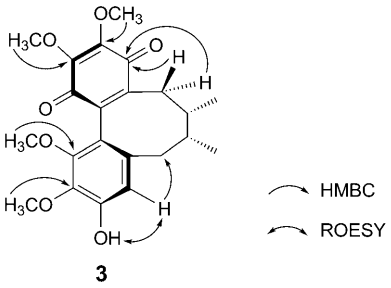
Fig. 2. *3D Structures of compound* **2** and **2a** *generated by computer modeling* (see *Exper. Part*) *and their key ROESY correlations*

and ROESY data (*Fig. 2*) established the structure of schisanhenone A (**2**) as (6*S*,7*R*,12a*S*)-5,6,7,8-tetrahydro-2,3,10,11,12-pentamethoxy-6,7-dimethyldibenzo[*a*,*c*]cyclooctene-1,4-dione.

The ^{1}H-NMR spectrum of **2** exhibited 2 *d* ($J = 7.0$ Hz) for two Me groups, as well as the resonances for two benzylic CH_2 and five MeO groups, indicating no change of the cyclooctene moiety as compared to that of **1**. The ^{1}H-NMR spectrum of **2** revealed the presence of only one aromatic-proton signal and no signal at δ(H) 5.0–6.0 for a phenolic OH group, while that of **1** showed signals for two aromatic protons and a phenolic OH group at δ(H) 5.72 (br. *s*). The IR spectrum of **2**, without absorption above 3000 cm^{-1}, further confirmed the absence of a phenolic OH group, and the absorptions at 1656 and 1608 cm^{-1} suggested the existence of two ketone groups, which was supported by the ^{13}C-NMR resonances at δ(C) 184.3 and 183.2. One ketone group was deduced to be at C(4) by the HMBCs of CH_2(5) (δ(H) 1.87 and 3.17)/C(4) (δ(C) 184.3) (*Fig. 1*). The HMBCs of the five MeO with C(2), C(3), C(10), C(11), and C(12) established the substitution sites of these MeO groups; the other ketone group was thus undoubtedly determined to be at C(1). A *p*-quinone structure was established and is a reasonable explanation for the absence of one aromatic proton and the phenolic OH group. The CD spectrum of **2** had a negative *Cotton* effect at 275 nm and a positive one at 222 nm, indicating that **2** contains an axially chiral (a*S*)-1,1′-biphenyl moiety ((*P*)-helicity), which is different from **1** [7]. A twist-boat (TB) conformation for the cyclooctene moiety of **2** was deduced by the ROESY correlations H–C(9)/H_α–C(8), H_α–C(8)/Me–C(6), Me–C(6)/H_α–C(5), and Me–C(6)/Me–C(7) [8]. The conclusion was further corroborated by the 3D molecular model of **2** (*Fig. 2*).

Compound **3**, obtained as a yellow amorphous solid, has the molecular formula $C_{22}H_{26}O_7$ as deduced from its HR-ESI-MS (*m*/*z* 403.1767 ($[M+H]^+$)). The ^{1}H-NMR spectrum of **3** (*Table 1*) was quite similar to that of **2**, except for the presence of a phenolic OH group (δ(H) 5.80 (br. *s*)) instead of an MeO group in **2**, which was

supported by the molecular mass fourteen units lower than that of **2**, as well as the absorption at 3418 cm^{-1} in the IR spectrum of **3**. Schisanhenone B (**3**) was finally identified as (6*S*,7*R*,12a*S*)-5,6,7,8-tetrahydro-10-hydroxy-2,3,11,12-tetramethoxy-6,7-dimethyldibenzo[*a*,*c*]cyclooctene-1,4-dione on the basis of further spectral data (^{13}C-NMR (*Table 2*), HMBC (*Fig. 3*), NOE and CD).

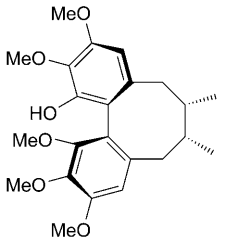


3

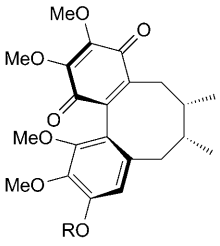
Fig. 3. *Key HMBC and ROESY correlations observed for* **3**

In the HMBC spectrum of **3** (*Fig.* 3), the cross-peaks of the four MeO signals (δ(H) 4.04, 4.04, 3.87, and 3.73) with δ(C) 144.1 (C(2)), 144.1 (C(3)), 136.8 (C(11)), and 149.3 (C(12)) established their connectivities. The OH group was thus positioned at C(10) (δ(C) 150.2), which was confirmed by the NOE correlation of the aromatic H–C(9) with the OH proton. Moreover, the chemical shifts for four MeO groups in the ^{13}C-NMR spectrum of **3** were around δ(C) 60, which was a further evidence in support of our deduction since an MeO group at C(10) would appear at δ(C) *ca.* 55, due to the shielding effect by the *o*-positioned H–C(9) [9–11]. The CD spectrum of **3** had a negative *Cotton* effect at 274 nm and a positive one at 219 nm, indicating that **3** contains an axially chiral (a*S*)-1,1′-biphenyl moiety ((*P*)-helicity) like the one in **2**. Similarly, **3** was assigned to have a cyclooctene moiety in a twist-boat (TB) conformation according to the ROESY experiment.

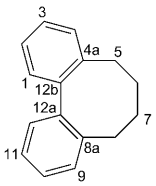
Metabolites **2** and **3** are considered of interest since their structures with a *p*-quinone unit are different from those of the naturally occurring dibenzocyclooctene lignans and metabolites of the analogues from the Schisandraceae plants [12–14]. The existence of a phenolic OH group and a proton in *p*-position to it in **1** should be the key structure for its quinonization in the *in vivo* metabolism, in analogy to a natural product with a similar structure [15]. The intestinal flora might be responsible for the



1



2 R = Me
3 R = H



metabolism, since a natural product with a *p*-positioned aromatic proton and a phenolic OH group failed to produce any obvious metabolites after incubation with rat-liver microsome or culture with hepatocyte, while its aerobatic incubation with the human intestinal bacterium *Klebsiella pneumoniae* resulted in the formation of a *p*-quinone metabolite which is just the main metabolite in the *in vivo* experiment with rats [16]. It was suggested that the intestinal flora plays an important role in this kind of metabolic pattern of natural phenolic compounds.

Interestingly, a product of (±)-deoxyschizandrin (=(6*RS*,7*SR*,12a*RS*)-5,6,7,8-tetrahydro-1,2,3,10,11,12-hexamethoxy-6,7-dimethyldibenzo[*a*,*c*]cyclooctene) treated with $[Fe(MeCN)_6]^{2+}/Ac_2O/H_2O_2$ was reported to have the same planar structure as **2**, and its biphenyl configuration is (12a*R*), the same as that of the corresponding natural lignan [17]. However, both metabolites obtained in our study have an inversed biphenyl configuration, as compared to that of schisanhenol (**1**). The change of biphenyl configuration was probably caused by the intestinal flora, due to its stereoselectivity in metabolism [18–20]; this needs to be established by further investigations. In addition, it is easily understood that **3** should be a demethylated metabolite of **2**, as such transformations commonly happen in the *in vivo* metabolism of dibenzocyclooctene lignans [12–14].

The isolated metabolites **2** and **3** were prone to change gradually to their conformational isomers **2a** and **3a** respectively in the solutions, until reaching a ratio of 1:1. The ^{1}H- and ^{13}C-NMR data of **2a** and **3a** (*Tables 1* and *2*) were assigned after picking out those of **2** and **3** in the spectra of the mixtures **2**/**2a** and **3**/**3a**. A twist-boat-chair (TBC) conformation for the cyclooctene moiety of **2a** was established by the ROESY correlations H–C(9)/Me–C(7), H–C(9)/H_α–C(8), H_α–C(5)/Me–C(7), and Me–C(6)/Me–C(7) [21], which was further supported by the 3D molecular model of **2a** (*Fig. 2*). Similarly, **3a** was assigned to have the same conformation as **2a**, according to the ROESY experiment.

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Experimental Part

General. Anal. and prep. TLC: silica gel plates (*GF_{254}*; *Yan-tai Institute of Chemical Technology*). Optical rotations (ORD): *Jasco-P-1020* spectropolarimeter. Melting points: *XT-4*-micro-melting-point apparatus (*Tai-ke Apparatus*); uncorrected. UV Spectra: *Shimadzu-UV-260* spectrophotometer; in anh. MeOH; λ_{max} (logε) in nm. CD Spectra: *Jasco-J-715* spectropolarimeter, λ ($\Delta\varepsilon$) in nm. IR Spectra: *Avatar-360-E.S.P.* spectrophotometer (*Thermo Nicolet*); as CH_2Cl_2 liquid membrane; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-400* spectrometer; in $CDCl_3$; δ in ppm, *J* in Hz. ESI-MS: *Agilent-1946D* mass spectrometry detector; in *m*/*z*. HR-ESI-MS: *Bruker-APEXIII-7.0-Tesla*-spectrometer.

Computer Modeling (*Fig. 2*). The modeling was performed with the SYBYL 6.9 software on a *Silicon-Graphics* workstation. The structure was optimized with the Tripos force-field.

Material. Schisanhenol (**1**) was isolated from the fruits of *Schisandra rubriflora* and identified by comparing its UV, IR, ^{1}H-NMR, and EI-MS data with those reported [22]. Its purity was determined to be more than 99% by HPLC analysis.

Animals and Administration. Animal studies were complied with the guidelines of the Animal Ethics Committee of Fudan University (Shanghai, China). All rats were supplied by the Laboratory Animal Center of Fudan University (certificate No. SCXK2002-0002 (Shanghai)). Six *Sprague-Dawley* rats (body weight 200–250 g) were fasted for 12 h before being dosed in metabolic cages with free access to water. Each of them was orally administered with 20 mg of **1** (dissolved in 2.2 ml of 45.5% EtOH) through a stomach gavage needle, and underwent the same procedure once more after a 12 h time lag.

Extraction and Isolation. The dried feces sample (40 g) was soaked with $CHCl_3$ (250 ml) for 3 d. The $CHCl_3$ extract was concentrated to yield a semisolid (2.2 g) which was suspended in H_2O (20 ml) and extracted with petroleum ether (3×20 ml). The combined org. phase was concentrated to yield a residue (400 mg). Prep. TLC (petroleum ether/acetone 3:1) of this residue yielded **2** (6 mg) and **3** (3 mg).

(6S,7R,12aS)-5,6,7,8-Tetrahydro-2,3,10,11,12-pentamethoxy-6,7-dimethyldibenzo[a,c]cyclooctene-1,4-dione (= *Schisanhenone A*; **2**): Reddish amorphous powder. M.p. 108–110°. $[\alpha]_D^{25} = +6.0$ ($c = 0.1$, MeOH). UV (MeOH): 215 (1.996), 276 (0.618). CD ($c = 0.005$, MeOH): 222 (+20), 272 (−10). IR: 2938, 2874, 1656, 1608, 1402, 1116, 1035, 735. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 417.2 ($[M+H]^+$). HR-ESI-MS: 439.1740 ($[M+Na]^+$, $C_{23}H_{28}NaO_7^+$; calc. 439.1727).

(6S,7R,12aS)-5,6,7,8-Tetrahydro-10-hydroxy-2,3,11,12-tetramethoxy-6,7-dimethyldibenzo[a,c]cyclooctene-1,4-dione (= *Schisanhenone B*; **3**): Yellow amorphous powder. M.p. 106–108°. $[\alpha]_D^{25} = +9.9$ ($c = 0.1$, MeOH). UV (MeOH): 211 (1.722), 274 (0.398). CD ($c = 0.005$, MeOH): 219 (+20), 274 (−15). IR: 3418, 2926, 2850, 1651, 1608, 1456, 1278, 1087, 736. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS: 403.1 ($[M+H]^+$). HR-ESI-MS: 403.1767 ($[M+H]^+$, $C_{22}H_{27}O_7^+$; calc. 403.1751).

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