Rubriflorin A and B, Two Novel Partially Saturated Dibenzocyclooctene Lignans from *Schisandra rubriflora*

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From the stems of *Schisandra rubriflora*, two novel partially saturated dibenzocyclooctene lignans, named rubriflorin A (**1**) and B (**6**), as well as the seven known partially saturated dibenzocyclooctene lignans kadsumarin A (**2**), kadsurin (**3**), heteroclitin B (**4**), heteroclitin C (**5**), heteroclitin D (**7**), interiorin (**8**), and interiorin B (**9**) were isolated. The structures of the new compounds **1** and **6** were established on the basis of spectral analysis as (5*R*,6*S*,7*R*,8*R*,13a*S*)-8-(acetyloxy)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethylbenz([3,4]cycloocta[1,2-*f*][1,3]benzodioxol-5-yl (2*Z*)-2-methylbut-2-enoate and (6*R*,7*R*,12a*S*)-7,8-dihydro-12-hydroxy-1,2,3,10,11-pentamethoxy-6,7-dimethyl-6*H*-dibenzo[*a*,*c*]cycloocten-5-one, respectively.

Introduction. – The genus *Schisandra* (Schisandraceae) is represented in China with 19 species, mostly growing in the southwestern part of the country on mountains 1500 m above sea level or higher [1]. *Schisandra rubriflora* RHED. et WILS has long been used as a tonic in Chinese folk medicine. It has been shown to be effective in reducing the level of GPT (glutamic-pyruvic transaminase) [2][3]. Previous investigations revealed the presence of lignans in the seeds of the plant [4]. As part of our studies of medicinal plants of the Schisandraceae family, we studied the lignans of the stem of *S. rubriflora*; two new partially saturated dibenzocyclooctene lignans, named rubriflorin A (**1**) and B (**6**), as well as seven known partially saturated dibenzocyclooctene lignans, were isolated. The known compounds were identified as kadsumarin A (**2**) [5], kadsurin (**3**) [6], heteroclitin B (**4**), heteroclitin C (**5**) [7], heteroclitin D (**7**) [7], interiorin (**8**) [8], and interiorin B (**9**) [9] (*Fig. 1*). Here we report the isolation and structure elucidation of **1** and **6**.

Results and Discussion. – Rubriflorin A (**1**) was isolated as optically active colorless needles. Its molecular formula was determined as $C_{30}H_{36}O_{10}$ by HR-EI-MS (*m*/*z* 556.2269 (M^+)). The IR spectrum showed characteristic absorptions for an α,β-unsaturated ester (1708 and 1648 cm^{-1}) and an aromatic ring (1599 and 1504 cm^{-1}). The UV absorptions at 218 (4.60), 253 (4.15), and 284 nm (3.78) suggested the presence of a biphenyl chromophore in which rotation of the phenyl ring is restricted by the condensed acyclic ring [10]. The CD curves of **1** showed a negative *Cotton* effect around 255 nm indicating an (*S*)-biphenyl configuration ((*P*) helicity) [10]. Based on further spectral data (^{1}H- and ^{13}C-NMR (*Table 1*), ^{1}H,^{1}H-COSY, HMQC, HMBC, NOESY, and MS) (*Fig. 2*), the structure of compound **1** was elucidated as

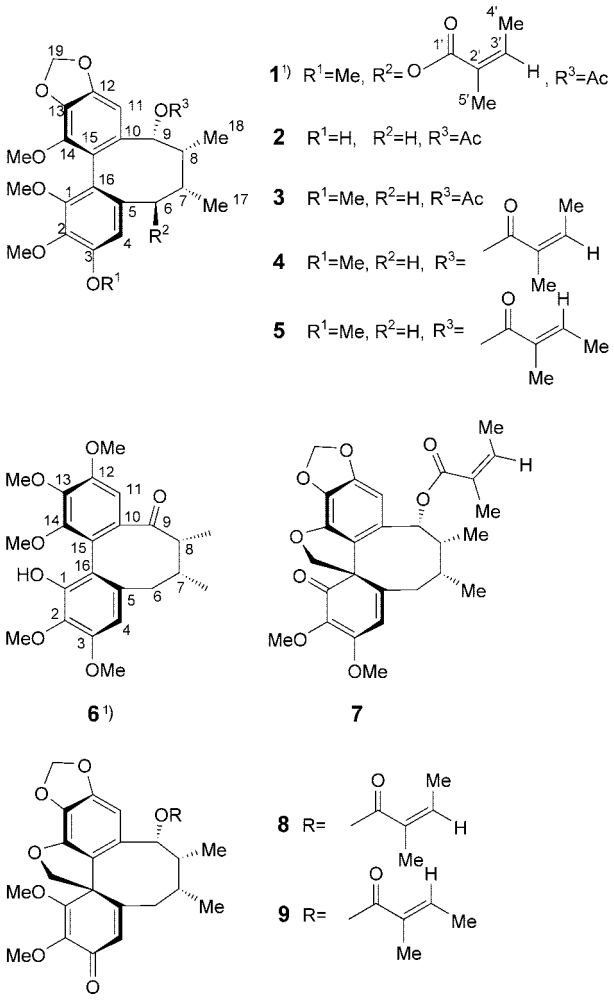

Fig. 1. *Structures of compounds* **1**–**8**

(5*R*,6*S*,7*R*,8*R*,13a*S*)-8-(acetyloxy)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethyl-benzo[3,4]cycloocta[1,2-*f*][1,3]benzodioxol-5-yl (2*Z*)-2-methylbut-2-enoate.

The ^{1}H-NMR spectrum of **1** showed signals due to two benzylic aromatic protons (δ 6.71 and 6.44 (each *s*)), an OCH_2O moiety (δ 5.96 and 5.95 (each *d*, $J = 1.2$ Hz), two CH–O moieties (δ 5.83 and 5.73 (each *d*, $J = 7.1$, 2.9 Hz)) and four MeO groups (δ 3.90, 3.88, 3.85, and 3.82 (each *s*)). The ^{13}C-NMR spectrum clearly indicated the presence of twelve C-atoms (C1 to C5, C(16) and C(10) to C(15), revealing a biphenyl moiety[1]). A butane-1,4-diyl moiety was predicted due to the prominent cross-peaks of H–C(6) (δ 5.83 (*d*, $J = 7.1$ Hz)), H–C(7) (δ 2.21 (*m*)), H–C(8) (δ 2.12 (*m*)) and H–C(9) (δ 5.73 (*d*, $J = 2.9$ Hz)) in the ^{1}H,^{1}H COSY plot. Moreover, the HMBC correlations (*Fig. 2*) H–C(6) (δ(H) 5.83)/C(4) (δ(C) 110.0) and C(16) (δ(C) 123.6) and H–C(9) (δ(H) 5.73)/C(11) (δ(C) 102.7) and C(15) (δ(C) 121.6) implied that **1** has a substituted butane-1,4-diyl part

[1]) Arbitrary numbering; for systematic names, see *Exper. Part*.

Table 1. *^1H-(500 MHz) and ^{13}C-NMR (125 MHz) Data of Rubriflorin A* (**1**) *in $CDCl_3$*. δ in ppm. Arbitrary numbering[1]).

	δ(H)	δ(C)		δ(H)	δ(C)
C(1)		152.0	C(16)		123.6
C(2)		141.7	Me(17)	0.94 (*d*, *J* = 6.9 Hz)	16.0
C(3)		152.2	Me(18)	1.03 (*d*, *J* = 6.9 Hz)	20.3
H–C(4)	6.71 (*s*)	111.0	CH_2(19)	5.96, 5.95 (*d*, *J* = 1.2 Hz)	101.4
C(5)		131.7	*Me*O–C(1)	3.57 (*s*)	61.0
H–C(6)	5.83 (*d*, *J* = 7.1 Hz)	81.1	*Me*O–C(2)	3.88 (*s*)	60.6
H–C(7)	2.21 (*m*)	39.1	*Me*O–C(3)	3.90 (*s*)	56.4
H–C(8)	2.12 (*m*)	39.1	*Me*O–C(14)	3.77 (*s*)	60.0
H–C(9)	5.73 (*d*, *J* = 2.9 Hz)	81.1	C(1′)		167.1
C(10)		133.5	C(2′)		128.2
H–C(11)	6.44 (*s*)	102.7	H–C(3′)	5.96 (*m*)	138.8
C(12)		149.0	Me(4′)	1.50 (*dq*, *J* = 1.5, 7.2 Hz)	16.0
C(13)		136.4	Me(5′)	1.86 (*dq*, *J* = 1.5, 2.9 Hz)	20.3
C(14)		142.2	MeCOO–C(9)		170.4
C(15)		121.6	*Me*COO–C(9)	1.59 (*s*)	21.0

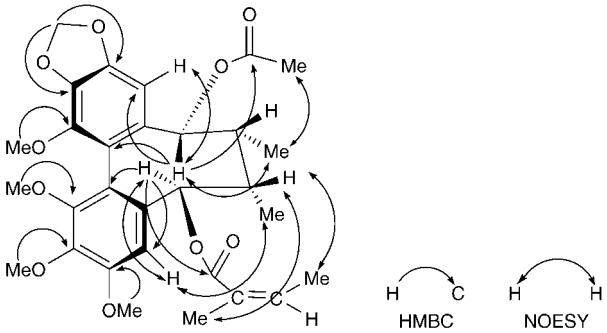

Fig. 2. *Key correlations in the HMBC and NOESY experiments of rubriflorin A* (**1**)

(C(6)–C(7)–C(8)–C(9)) at C(5) and C(10), and two substituted aromatic moieties linked between C(15) and C(16). From the above evidence, the skeleton of **1** was deduced to be a substituted hexahydrodibenzocyclooctene lignan similar to those isolated previously from Schisandraceae plants [11][12].

The substituents appearing in the ^{1}H- and ^{13}C-NMR spectra of **1** were assigned as MeO–C(1), MeO–C(2), MeO–C(3), MeO–C(14), OCH_2(19)O, MeCl–(17), and Me–Cl(18), based on the HMQC and HMBC spectra. An angeloyl (=(2*Z*)-2-methylbut-2-enoyl) group (H–C(3′), Me(4′), Me(5′), C(1′) to C(5′)) and an acetic acid ester (MeCOO) were also present (see NMR data in *Table 1*). Besides the molecular ion at *m*/*z* 556, the EI-MS of **1** showed two characteristic peaks at *m*/*z* 457 ($[M - C_5H_7O_2]^+$) and 497 ($[M - AcO]^+$) confirming the presence of an angeloyloxy and an acetyloxy group. The HMBC correlations (see *Fig. 2*) H–C(6) (δ(H) 5.83)/C(1′) (δ(C) 167.1) and H–C(9) (δ(H) 5.73)/MeCOO (δ(C) 170.4) suggested that the angeloyloxy group is attached at C(6) and the acetyloxyl group at C(9). In addition, the $J(H_\beta–C(8), H_\beta–C(9))$ value of 2.9 Hz and the NOEs (*Fig. 2*) between H–C(4)/Me(17), H–C(4)/H_a–(6), H–C(9)/H–C(11) and H–C(9)/Me(18) indicate a twist-boat-chair conformation of the cyclooctadiene moiety [11].

Rubriflorin B (**6**) was isolated as yellowish powder. Its molecular formula was determined as $C_{23}H_{28}O_7$ by HR-EI-MS (*m*/*z* 416.4736 (M^+)). The IR spectrum contained characteristic absorptions for an OH group (3384 cm^{-1}, (br.)), a carbonyl

group (1662 cm^{-1}), and an aromatic ring (1586 and 1507 cm^{-1}). The UV spectrum showed λ_{max} at 222 (4.80), 254 (4.25), and 285 nm (3.98), characteristic of a biphenyl chromophore. The CD curves of **6** also showed a negative *Cotton* effect around 253 nm indicating an (*S*)-biphenyl configuration [10]. Based on further spectral data (^{1}H- and ^{13}C-NMR (*Table 2*), ^{1}H,^{1}H-COSY, HMQC, and HMBC), the structure of compound **6** was elucidated as (6*R*,7*R*,12a*S*)-7,8-dihydro-12-hydroxy-1,2,3,10,11-pentamethoxy-6,7-dimethyl-6*H*-dibenzo[*a*,*c*]cycloocten-5-one.

Table 2. *^{1}H-*(500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Rubriflorin B* (**2**) *in $CDCl_3$.* δ in ppm. Arbitrary numbering[1]).

	δ(H)	δ(C)		δ(H)	δ(C)
C(1)		152.5	C(12)		134.3
C(2)		133.9	C(13)		151.5
C(3)		152.3	C(14)		146.1
H–C(4)	6.38 (*s*)	102.8	C(15)		124.1
C(5)		147.4	C(16)		115.8
CH_2(6)	2.64 (*dd*, *J* = 12.9, 6.6 Hz),	40.2	Me(17)	0.83 (*d*, *J* = 6.9 Hz)	15.4
	2.22 (*dd*, *J* = 12.3, 12.6 Hz)		Me(18)	1.01 (*d*, *J* = 6.6 Hz)	15.3
H–C(7)	1.82 (*m*)	41.4	*Me*O–C(2)	3.95 (*s*)	60.9
H–C(8)	2.69 (*qd*, *J* = 6.6, 3.8 Hz)	44.6	*Me*O–C(3)	3.93 (*s*)	55.9
C(9)		200.9	*Me*O–C(12)	3.92 (*s*)	55.8
C(10)		135.9	*Me*O–C(13)	3.56 (*s*)	61.2
H–C(11)	7.63 (*s*)	108.1	*Me*O–C(14)	3.94 (*s*)	60.8

The ^{1}H-NMR spectrum of **6** showed signals due to two aromatic protons (δ 7.63, 6.38 (2*s*), an OH proton (δ 5.90, 5.95 (2*s*), and five MeO groups (δ 3.95, 3.94, 3.93, 3.92, 3.56 (each *s*). The ^{13}C-NMR spectrum clearly indicated the presence of twelve C-atoms (C(1) to C(5), C(16) and C(10) to C(15), revealing a biphenyl moiety. A propane-1,3-diyl moiety was predicted due to the prominent cross-peaks of CH_2(6) (δ 2.64 (*dd*, *J* = 12.9, 6.6 Hz) and 2.22 (*dd*, *J* = 12.3, 12.6 Hz)), H–C(7) (δ 1.82 (*m*)), and H–C(8) (δ 2.69 (*qd*, *J* = 6.6, 3.8 Hz)) in the ^{1}H,^{1}H COSY plot. Moreover, the obvious HMBC correlations CH_2(6) (δ(H) 2.64, 2.22)/C(4) (δ(C) 102.8), C(10) (δ(C) 108.1) and C(16) δ(C) 115.8)/H–C(8) (δ(H) 2.69 and H–C(7) (δ(H) 1.82)/C(9) (δ(C) 200.9), and H–C(11) (δ(H) 7.63)/C(9) (δ(C) 200.9) implied that **6** possesses a substituted butane-1,4-diyl moiety (C(6)–C(7)–C(8)–C(9)) at C(5) and C(10), and two substituted aromatic moieties connected at C(15) and C(16) linkage. From the above evidence, the skeleton of **6** was also deduced to be a substituted partially saturated dibenzocyclooctene lignan. The substituents were assigned as MeO–C(2), MeO–C(3), MeO–C(12), MeO–C(13), MeO–C(14), Me(17), and Me(18) based on the ^{1}H,^{1}H COSY, HMQC, and HMBC spectra.

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Experimental Part

*General. M*elting points (uncorrected): *XT-4* melting-point apparatus. $[\alpha]_D$ Values: *Jasco 20C* digital polarimeter. CD Curves: *Jasco J500A* dichrograph; ($\Delta\varepsilon$) in nm. UV Spectra: *UV-210A* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker Avance DRX-500* instrument; $SiMe_4$ as internal reference, δ in ppm, *J* in Hz. EI-MS: *VG Auto-Spec-3000* mass spectrometer; in *m*/*z* (rel.%).

Plant Material. The stems of *S. rubriflora* RHED. et WILS were collected in Linchang Country, Yunnan Province, P.R. China, in April 2001. The plant was identified by Prof. *Zhi-hao Hu*, Department of Biology, School of Life Science, Yunnan University. A voucher specimen (No. 01-003) was deposited in the Department of Phytochemistry, School of Pharmacy, Yunnan University, Yunnan, P.R. China.

Extraction and Isolation. The stem of *Schisandra rubriflora* (6.0 kg) was air-dried, ground, and extracted (3 ×) with 95% EtOH at r.t. The EtOH extract was evaporated to yield a residue, which was suspended in H_2O and then partitioned with $CHCl_3$, AcOEt, and BuOH, successively. The AcOEt extract (60 g) was subjected to column chromatography (CC; silica gel, petroleum ether/Me_2CO 60:1 → to 0:1): *Fractions* (*1–8*). *Fr. 2* was further purified by CC (silica gel, petroleum ether/Et_2O 20:1 → 1:1) as eluent and finally by reversed-phase CC (C_{18} silica gel, MeOH/H_2O 8:2 → 1:1): **1** (56 mg), **8** (210 mg), and **9** (65 mg). *Fr. 3* was purified by CC silica gel, cyclohexane/^{i}PrOH 40:1 → 1:1); further reversed-phase CC (C_{18} silica gel) MeOH/H_2O 8:2 → 2:8) gave **4** (60 mg), **5** (85 mg), **6** (25 mg) and **7** (110 mg). *Fr. 5* was subjected to CC (silica gel, petroleum ether/AcOEt (10:1 → 0:1): **2** (1.045 g) and **3** (875 mg).

Rubriflorin A (= *(5*R*,6*S*,7*R*,8*R*,13a*S*)-8-(Acetyloxy)-5,6,7,8-tetrahydro-1,2,3,13-tetramethoxy-6,7-dimethylbenzo[3,4]cycloocta[1,2-f][1,3]benzodioxol-5-yl (2*Z*)-2-Methylbut-2-eonate*; **1**): Colorless needles ($CHCl_3$). M.p. 176–178°. $[\alpha]_D^{26} = +62.99$ ($c = 0.254$, $CHCl_3$). CD (MeOH, $c = 0.0005$ g/ml): 255 (−45000), 300 (−3000). UV ($CHCl_3$): 218 (4.60), 253 (4.15), 284 (3.78). IR (KBr): 3436, 1708, 1648, 1599, 1504, 1477, 974, 768. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 556(100), 497(22), 457(39), 397(10), 357(28), 329(6), 159(4), 97(4), 83(72). HR-EI-MS: 556.2269 ($C_{30}H_{36}O_{10}^+$, M^+; calc. 556.2308).

Rubriflorin B (= *(6*R*,7*R*,12a*S*)-7,8-dihydro-12-hydroxy-1,2,3,10,11-pentamethoxy-6,7-dimethyl-6*H*-dibenzo[*a*,*c*] cycloocten-5-one*; **6**): Yellowish powder ($CHCl_3$). M.p. 175–176°. $[\alpha]_D^{19} = -32.68$ ($c = 0.153$, $CHCl_3$). CD (MeOH, $c = 0.0005$ g/ml): 253 (−23400), 298 (−2000). UV ($CHCl_3$): 222 (4.80), 254 (4.25), 285 (3.98). IR (KBr): 3384, 3162, 297, 1662, 1586, 1507, 1488, 993, 823. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 416(100), 401(8), 385(9), 360(12), 344(14), 312(10), 284(12), 257(4), 249(5), 164(11), 157(10), 115(5), 83(6). HR-EI-MS: 416.4736 ($C_{30}H_{36}O_{10}^+$, M^+; calc. 416.4704).

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