A Novel Norlignan and a Novel Phenylpropanoid from *Peperomia tetraphylla*

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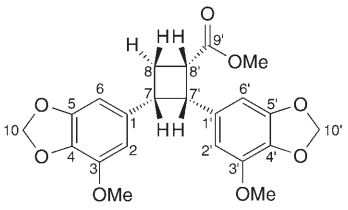
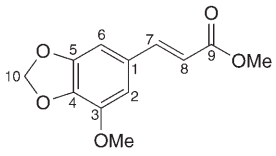
A novel cyclobutane-type norlignan, peperotetraphin (= methyl *rel*-(1*R*,2*S*,3*S*)-2,3-bis(7-methoxy-1,3-benzodioxol-5-yl)cyclobutanecarboxylate; **1**), and a novel phenylpropanoid, *i.e.*, methyl (2*E*)-3-(7-methoxy-1,3-benzodioxol-5-yl)prop-2-enoate (**2**), along with three known compounds, α-asarone (= 1,2,4-trimethoxy-5-[(1*E*)-prop-1-en-1-yl]benzene), vanillic acid (= 4-hydroxy-3-methoxybenzoic acid), and veratric acid (= 3,4-dimethoxybenzoic acid), were isolated from the EtOH extract of the whole plant of *Peperomia tetraphylla*. Their structures were determined by spectroscopic methods, especially 1D- and 2D-NMR techniques. This is the first report of naturally occurring cyclobutane-type norlignans.

Introduction. – *Peperomia tetraphylla* (FORST. F.) HOOKER. *et* ARNOTT. (*Piperaceae*), a perennial herb grown widely in Southern China, is an effective folk medicine to treat cough, asthma, beriberoid disease, dysenteria, diarrhea, and agrypnia [1]. From the other plants of the genus *Peperomia*, a variety of structurally diversified compounds including lignans [2], polyketides [3], benzopyranones [4], benzopyrans [5], quinones [6], and phenylpropanoids [7] were reported. But, there is no phytochemical study on this species. In the current study, a novel cyclobutane-type norlignan, named peperotetraphin (**1**)[1]), and the novel phenylpropanoid **2**[1]), along with three known compounds, were isolated from the whole plant. Herein, we describe the isolation and structural elucidation of the two new compounds.



1[1])



2[1])

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

Results and Discussion. – Compound **1** was obtained as a colorless gum. The molecular formula was deduced to be $C_{22}H_{22}O_8$ from the HR-ESI-MS data (*m/z* 453.2309 ($[M + K]^+$)). The strong IR absorptions at 1730, 1632, 1510, and 1431 cm^{-1} showed the presence of a carbonyl group and aromatic moieties. The structure and relative configuration of **1**, *i.e.*, (7β,7′β,8′β), were established by ^{1}H- and ^{13}C-NMR (*Table*) and 2D-NMR data.

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 600 and 150 MHz, resp.) *of* **1** *and* **2**. δ in ppm, *J* in Hz. Trivial atom numbering.

	1[1])		**2**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	136.6	–	129.2
H–C(2)	6.45 (*d*, *J* = 1.2)	100.9	6.71 (*s*)	109.2
C(3)	–	143.5[a])	–	143.7
C(4)	–	134.0	–	137.4
C(5)	–	149.0[a])	–	149.4
H–C(6)	6.38 (*d*, *J* = 1.2)	106.4[a])	6.76 (*s*)	101.3
H–C(7)	3.30–3.36 (*m*)	44.1	7.55 (*d*, *J* = 15.6)	144.6
H_α–C(8)	2.27–2.33 (*m*)	29.5	6.28 (*d*, *J* = 15.6)	116.3
H_β–C(8)	2.52–2.59 (*m*)		–	–
C(9)	–	–	–	167.5
C(1′)	–	137.8	–	–
H–C(2′)	6.46 (*d*, *J* = 1.2)	100.7	–	–
C(3′)	–	143.5[a])	–	–
C(4′)	–	133.9	–	–
C(5′)	–	149.0[a])	–	–
H–C(6′)	6.39 (*d*, *J* = 1.2)	106.4[a])	–	–
H–C(7′)	3.59–3.65 (*m*)	51.4	–	–
H–C(8′)	3.04–3.11 (*m*)	41.6	–	–
C(9′)	–	174.4	–	–
CH_2(10)	5.92[a]) (*s*)	101.3[a])	6.03	101.9
CH_2(10′)	5.92[a]) (*s*)	101.3[a])	–	–
MeO–C(3)	3.87 (*s*)	56.7[a])	3.92	56.6
MeO–C(3′)	3.88 (*s*)	56.7[a])	–	–
MeO–C(9′)	3.74 (*s*)	51.9	–	–
MeO–C(9)	–	–	3.79	51.6

[a]) Overlapped signals.

In the ^{1}H-NMR spectrum of **1** signals for two 1,3,4,5-tetrasubstituted benzene rings (δ 6.45 and 6.38, and 6.46 and 6.39 (each *d*, *J* = 1.2 Hz)), 2 OCH_2O (δ 5.92 (*s*)), 3 MeO (δ 3.88, 3.87, and 3.74 (each *s*)), as well as for five aliphatic protons (δ 3.59–3.65, 3.30–3.36, 3.04–3.11, 2.52–2.59, and 2.27–2.33) were observed. The ^{13}C-NMR spectrum of **1** showed 22 C-signals to which 12 aromatic C-atoms of two benzene rings, three C-atoms of the MeO groups, a carbonyl C-atom, and two methylenedioxy groups were attributed. Therefore, a four-membered ring was deduced to fulfill the requirement of the unsaturation degree and to take into account the four remaining C-atoms. The ^{1}H,^{1}H-COSY and HSQC data also disclosed a cyclobutane unit (*Fig. 1*). The HMBC correlations established the skeletal structure of **1** as shown in *Fig. 1* and the relationships of the protons of the cyclobutane unit with the two benzene rings and the carbonyl group. Furthermore, the HMBC correlations confirmed the assignments of

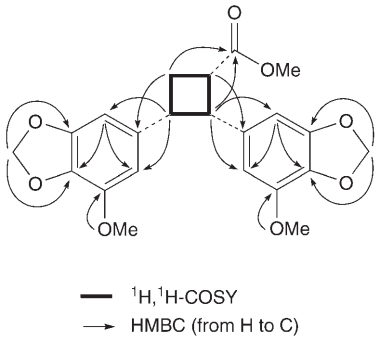


Fig. 1. *Selected ^{1}H,^{1}H-COSY and HMBC correlations of* **1**

2 OCH_2O and 3 MeO groups. The relative configuration of **1** was established on the basis of a NOESY experiment. In the NOESY plot, the NOE cross-peaks H–C(7)/H–C(7′), H–C(7′)/H–C(8′), H_β–C(8) (δ 2.52–2.59)/H–C(7), H–C(7′), and H–C(8′), H_α–C(8) (δ 2.27–2.33)/H–C(2) and H–C(6) demonstrated the *cis*-orientation of the proton(s) at C(7), C(7′), C(8′), and H_β–C(8).

Compound **2** was obtained as a colorless gum. Its molecular formula $C_{12}H_{12}O_5$ was deduced from the HR-ESI-MS data (*m*/*z* 259.0599 ($[M + Na]^+$)). The IR absorptions at 1715, 1635, 1509, and 1432 cm^{-1} showed the presence of a carbonyl group and an aromatic moiety. The ^{1}H- and ^{13}C-NMR (*Table*) and 2D-NMR data of **2** suggested that it possesses the structure of methyl (2*E*)-3-(7-methoxy-1,3-benzodioxol-5-yl)prop-2-enoate.

The ^{1}H- and ^{13}C-NMR and HSQC data of **2** indicated that the two aromatic protons at δ(H) 6.71 (*s*, 1 H) and 6.76 (*s*, 1 H) belonged to an 1,3,4,5-tetrasubstituted benzene ring, with the corresponding C-signals observed at δ(C) 129.2, 109.2, 143.7, 137.4, 149.4, and 101.3. In addition, signals for an OCH_2O (δ(H) 6.03(*s*); δ(C) 101.9), 2 MeO (δ(H) 3.92 and 3.79 (each *s*); δ(C) 56.6 and 51.6), as well as for two *trans*-positioned olefinic protons (δ(H) 6.28 and 7.55 (each *d*, $J = 15.6$ Hz); δ(C) 116.3 and 144.6) also were observed. The HMBC correlations established the skeletal structure of **2** as shown in *Fig. 2*.

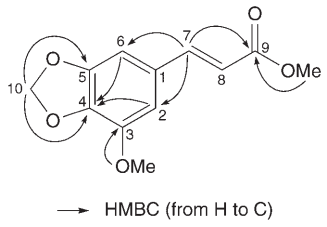


Fig. 2. *Selected HMBC correlations of* **2**

Because of the isolation of compound **2**, we believe that peperotetraphin (**1**) might be biosynthesized from compound **2** through a [2 + 2] cycloaddition reaction, followed by the loss of the COOMe group from the dimer. Also, the relative configuration of peperotetraphin (**1**) is in agreement with such a biogenetic hypothesis [8][9]. Up to date, this is the first report of a naturally occurring cyclobutane-type norlignan.

The three known compounds, α-asarone (=1,2,4-trimethoxy-5-[(1*E*)-prop-1-en-1-yl]benzene) [10], vanillic acid (=4-hydroxy-3-methoxybenzoic acid) [11], and veratric acid (= 3,4-dimethoxybenzoic acid) [12] were identified by comparison of their ^{1}H- and ^{13}C-NMR as well as ESI-MS data with those reported in the literatures. All of these known compounds were isolated from the title plant for the first time.

Experimental Part

General. All solvents used were of anal. grade (*Tianjin Chemical Plant,* Tianjin, P. R. China). Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Ocean Chemical Industry Co.*, China) or *Sephadex LH-20* (*Amersham Biosciences*). Optical rotation: *Perkin-Elmer 241* polarimeter. UV Spectra: *Shimadzu UV-2210*-UV/VIS spectrometer; $CHCl_3$ solns.; λ_{max} in nm. IR Spectra: *Vector 22-FTIR* spectrometer; $CHCl_3$ solns.; in cm^{-1}. NMR Spectra (^{1}H-, ^{13}C-, ^{1}H,^{1}H-COSY, HSQC, HMBC, DEPT, and NOESY): *Bruker AV-600* spectrometer; at 600 (^{1}H) or 125 MHz (^{13}C)); $CDCl_3$ solns.; δ in ppm rel. to $SiMe_4$, *J* in Hz. HR-ESI-MS: *Bio TOF-IIIQ* mass spectrometer; in *m*/*z*.

Plant Material. The whole plant of *Peperomia tetraphylla* was collected from Sichuan province, P. R. China, in November 2006, and was identified by Mr. *Wen-jin Zhang.* A voucher specimen (No. 2006-11L) was deposited in the West China School of Pharmacy, Sichuan University, P. R. China.

Extraction and Isolation. Dried whole plants (10 kg) were extracted with 95% EtOH (3×10 l) under reflux, and yielded *ca.* 1000 g of residue after evaporation of the solvent. The residue was suspended in H_2O and successively partitioned with petroleum ether, AcOEt, and BuOH to afford 55 g of petroleum ether extract, 100 g of AcOEt extract, and 45 g of BuOH extract. The AcOEt extract was subjected to CC (SiO_2, petroleum ether/acetone $40:1 \rightarrow 0:1$): *Fractions 1–10*. *Fr. 5* (17 g) was purified by repeated CC (SiO_2, cyclohexane/AcOEt $40:1 \rightarrow 2:1$; *Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **2** (3 mg) and α-asarone (100 mg). *Fr. 6* (4 g) was purified by repeated CC (SiO_2, petroleum ether/acetone $20:1 \rightarrow 2:1$; *Sephadex LH-20*, MeOH): **1** (7 mg). *Fr. 7* (4 g) was purified by repeated CC (SiO_2, petroleum ether/acetone $10:1 \rightarrow 0:1$; *Sephadex LH-20*, MeOH) to afford vanillic acid (18 mg) and veratric acid (12 mg).

Peperotetraphin (= *Methyl* rel-*(1*R*,2*S*,3*S*)-2,3-Bis(7-methoxy-1,3-benzodioxol-5-yl)cyclobutanecarboxylate*; **1**): Colorless gum. $[\alpha]_D^{20} = \pm 0$ ($c = 0.055$, $CHCl_3$). UV ($CHCl_3$): 252, 275. IR ($CHCl_3$): 2923, 2852, 1730, 1632, 1510, 1452, 1431, 1362, 1319, 1200, 1141, 1093, 1046, 967, 929, 827, 745, 720. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 453.2309 ($[M + K]^+$, $C_{22}H_{22}O_8K^+$; calc. 453.2298).

*Methyl (2*E*)-3-(7-Methoxy-1,3-benzodioxol-5-yl)prop-2-enoate* (**2**): Colorless gum. UV ($CHCl_3$): 249.5, 289.5, 324.5. IR ($CHCl_3$): 2923, 2852, 1715 (C=O), 1635, 1509, 1454, 1432, 1363, 1280, 1170, 1137, 1093, 1043, 976, 829. ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS: 259.0599 ($[M + Na]^+$, $C_{12}H_{12}O_5Na^+$; calc. 259.0583).

REFERENCES

[1] 'Dictionary of Chinese Traditional Medicines', Jiangsu New Medical College, Shanghai Scientific and Technologic Press, Shanghai, 1986, p. 1045.

[2] N. Li, J. L. Wu, J. Sakai, M. Ando, *J. Nat. Prod.*, **2003**, *66*, 1421.

[3] M. J. Cheng, S. J. Lee, Y. Y. Chang, S. H. Wu, I. L. Tsai, B. Jayaprakasam, I. S. Chen, *Phytochemistry* **2003**, *63*, 603.

[4] J. A. Mbah, M. H. K. Tchuendem, P. Tane, O. Sterner, *Phytochemistry* **2002**, *60*, 799.

[5] N. P. Seeram, H. Jacobs, S. McLean, W. F. Reynolds, *Phytochemistry* **1998**, *49*, 1389.

[6] V. Mahiou, F. Roblot, R. Hocquemiller, A. Cave, A. R. D. Arias, A. Inchausti, G. Yaluff, A. Fournet, *J. Nat. Prod*. **1996**, *59*, 694.

[7] J. C. Bayma, M. S. P. Arruda, A. H. Müller, A. C. Arruda, W. C. Canto, *Phytochemistry* **2000**, *55*, 779.

[8] M. Kuroyanagi, Y. Yamamoto, S. Fukushima, A. Ueno, T. Noro, T. Miyase, *Chem. Pharm. Bull.* **1982**, *30*, 1602.

[9] J. J. Tan, C. H. Tan, Y. Q. Wang, S. H. Jiang, D. Y. Zhu, *Helv. Chim. Acta* **2006**, *89*, 117.
[10] F. Zhang, Q. Xu, S. P. Fu, H. B. Xiao, X. M. Liang, *Chin. Tradit. Herbal Drugs* **2004**, *35*, 1215.
[11] Z. H. Yuan, L. J. Han, F. Xiao, S. Li, D. Y. Shi, J. Sun, M. Ma, Y. C. Yang, J. G. Shi, *China J. Chin. Mater. Med.* **2006**, *31*, 1790.
[12] J. Zheng, D. S. Zhao, B. Wu, L. J. Wu, *China J. Chin. Mater. Med.* **2002**, *27*, 749.

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