Rotundifolides A and B, Two New Enol-Derived Butenolactones from the Bark of *Litsea rotundifolia* var. *oblongifolia*

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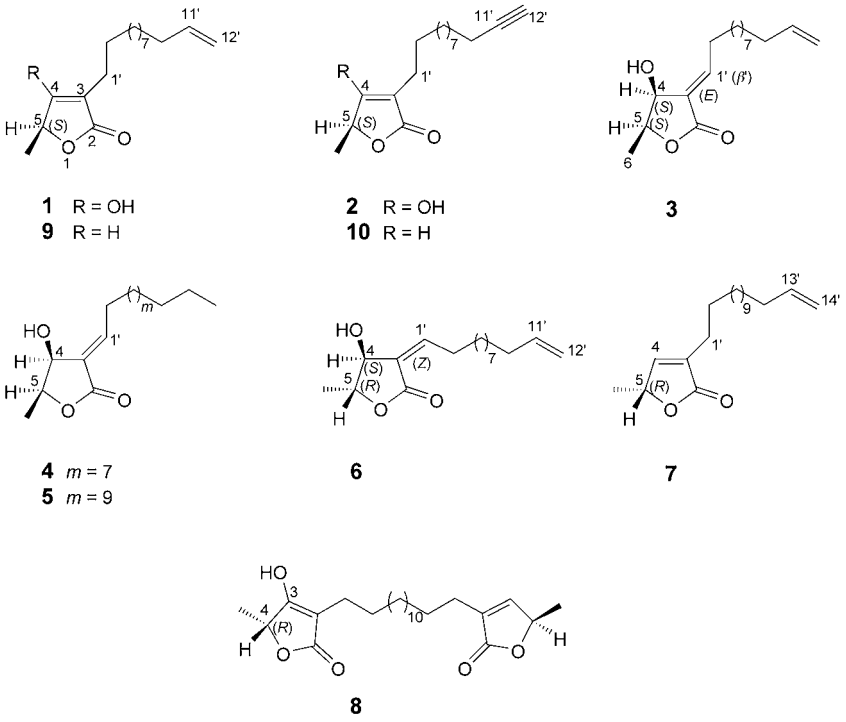
Two new enol-derived butenolactones, rotundifolides A (**1**) and B (**2**), along with four known related compounds, lincomolide C (**3**), lincomolide A (**4**), marliolide (**5**), and litsenolide A_1 (**6**), were isolated from the bark of *Litsea rotundifolia* var. *oblongifolia*. The structures of the new metabolites were characterized by spectroscopic methods and comparison with known compounds.

Introduction. – *Litsea* is a genus in the family Lauraceae with *ca.* 72 species distributed in South and Southwest China [1]. Most *Litsea* plants contain alkaloids [2–4], flavonoids [5][6], terpenes [7][8], lactones [9], and volatile oil [10], which were reported to possess a variety of biological activities ranging from antimicrobial, hypothermic, to antitumor activities [2][11][12]. In continuation of our work on the chemical constituents of Chinese medicinal plants, we have examined the secondary metabolites present in the bark of *L. rotundifolia* var. *oblongifolia*, since no phytochemical investigation has been done on the species. Two new enol-derived buteno-4-lactones, named rotundifolides A (**1**) and B (**2**), along with four known related butenolactones, lincomolide C (**3**) [13], lincomolide A (**4**) [14], marliolide (**5**) [15], and litsenolide A_1 (**6**) [16] were isolated from the title plant. This paper describes the structure elucidation of the new compounds.

Results and Discussion. – Specimens of *L. rotundifolia* var. *oblongifolia* were extracted exhaustively with MeOH, and the MeOH extract was partitioned between H_2O and organic solvents to afford AcOEt-soluble and BuOH-soluble fractions. The AcOEt extract was further separated by column chromatography and semi-prep. HPLC (see *Exper. Part*) to give the known metabolites **3**–**6** [13–16] and the new compounds **1** and **2**. The new compounds demonstrated considerable spectral analogy with the co-occurring known butenolides **3**–**6** [13–16].

Rotundifolide A (**1**), a colorless and optically active oil, exhibited the absorption bands of an OH group (3399 cm^{-1}) and an α,β-unsaturated γ-lactone (1747 and 1660 cm^{-1}) in the IR spectrum. The molecular formula of **1** was determined to be $C_{17}H_{28}O_3$ from its HR-EI-MS (*m*/*z* 280.2042 (M^+)). From analysis of the ^1H- and ^{13}C-NMR (*Table*) and UV spectra, compound **1** has the same unsaturated β-hydroxy-γ-methyl-γ-lactone structure as that of co-occurring lincomolide C (**3**) [13]. Nevertheless, the NMR spectra of **1** were somewhat different from those of **3**. On the basis of the spectral evidence, structure **1** was determined to be (5*S*)-3-(dodec-11-enyl)-4-hydroxy-



5-methylfuran-2(*5H*)-one, which was further confirmed by ^{1}H,^{1}H COSY, HMQC, and HMBC (*Fig.*) experiments.

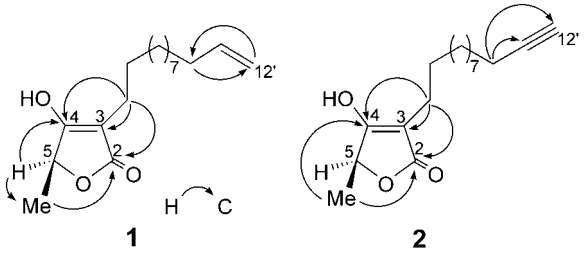


Figure. *Selected key HMBC correlations of* **1** *and* **2**

Careful comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of **3** revealed that the differences were mainly in the butenolactone moiety. The lack of the characteristic olefinic-proton resonance (H–C(1′)) and the H–C(4) resonance of **3** together with the appearance of a quaternary C-atom at δ 177.6 strongly indicated the enol-derived nature of the butenolactone moiety of **1**. In addition, the ^{1}H-NMR signals at δ 4.83 (*m*, H–C(5)) and 1.50 (*d*, $J = 6.0$ Hz, Me–C(5)) were attributable to a CH bearing a Me group. The presence of an endocyclic butenolactone moiety in **1** was further confirmed by the comparison of ^{1}H- and ^{13}C-NMR data of **1** with those of model compound **8** [17]. The absolute configuration at C(5) of **1** was suggested to be (*S*), opposite to that established for a related compound **7**, by comparing the positive $[\alpha]_D$ value of **1** ($[\alpha]_D = +11$ (dioxane)) with the

Table. *Selected ^{1}H- and ^{13}C-NMR Data*[a])[b]) *of Compounds* **1**–**3**

	1		**2**		**3**	
	δ(H)[b])	δ(C)[c])	δ(H)[b])	δ(C)[c])	δ(H)[b])	δ(C)[c])
C(2)	–	177.8 (*s*)	–	176.9 (*s*)	–	169.8 (*s*)
C(3)	–	100.7 (*s*)	–	101.0 (*s*)	–	130.5 (*s*)
C(4) or H–C(4)	–	177.6 (*s*)	–	176.6 (*s*)	4.80 (*d*, *J* = 4.9)	67.8 (*d*)
H–C(5)	4.83 (*m*)	75.3 (*d*)	4.82 (*m*)	74.9 (*d*)	4.52 (*dq*, *J* = 6.6, 4.9)	78.6 (*d*)
Me–C(5)	1.50 (*d*, *J* = 6.0)	17.8 (*q*)	1.49 (*d*, *J* = 6.0)	17.8 (*q*)	1.44 (*d*, *J* = 6.6)	14.0 (*q*)
H–C(1′) or CH_2(1′)	2.19 (*t*, *J* = 6.1)	31.8 (*t*)	2.18 (*m*)	31.9 (*t*)	6.94 (*t*, *J* = 7.8)	147.8 (*d*)
CH_2(2′)	–	–	–	–	2.39 (*dt*, *J* = 7.8)	32.0 (*t*)
CH_2(10′)	2.02 (*m*)	33.7 (*t*)	2.18 (*m*)	18.3 (*t*)	2.01 (*m*)	33.8 (*t*)
C(11′) or H–C (11′)	5.79 (*ddt*, *J* = 6.6, 10.2, 17.0)	139.1 (*d*)	–	84.7 (*s*)	5.78 (*ddt*, *J* = 6.6, 10.2, 17.0)	139.2 (*d*)
H–C (12′) or CH_2(12′)	4.98 (*dd*, *J* = 1.6, 17.0), 4.92 (br. *d*, *J* = 10.2)	114.0 (*t*)	1.93 (*s*)	68.0 (*d*)	4.97 (*dd*, *J* = 1.7, 17.0), 4.91 (br. *d*, *J* = 10.2)	114.1 (*t*)

[a]) *Bruker DRX-400* spectrometer; measured in $CDCl_3$; chemical shifts in δ relative to the $CDCl_3$ (δ 77.0) and residual $CHCl_3$ signals (δ 7.26) as internal standard, respectively; assignments by analysis of 1D and 2D NMR spectra. [b]) Unassigned CH_2 protons contributed to an intense signal at δ 1.25–1.30. [c]) The CH_2 C-atoms not assigned specifically contributed to an intense signal centered at δ 29.8.

negative one of **7** ($[\alpha]_D = -29.8$ (dioxane)) [18]. Moreover, the same positive $[\alpha]_D$ sign of **1** as those of the closely related compounds **9** and **10** [19] further supported the assigned (5*S*) configuration.

Rotundifolide B (**2**), a colorless oil, gave a quasi-molecular ion at *m*/*z* 277 ($[M-H]^-$) in its ESI-MS, corresponding to a molecular formula $C_{17}H_{26}O_3$, of two mass units less than that of **1**. The ^{1}H-NMR spectrum of **2** exhibited similarities with that of compound **1**. In fact, compound **2** differs from **1** only by the presence of a terminal ethynyl group (δ(H) 1.93 (*s*, H–C(12′)), δ(C) 68.0 (*d*) and 84.7 (*s*)) instead of a terminal C=C bond in the C_{12} side chain. As in the case of **1**, the configuration at C(5) was assigned to be (*S*).

The new enol-derived butenolactones **1** and **2** are encountered for the first time in Lauraceae plants although their corresponding deoxy derivatives **9** and **10** have been isolated from the leaves of three species of *Hortonia* (family Monimiaceae) very recently [19]. In particular, it is of interest that both **9** and **10** were reported to have potent mosquito larvicidal activity against the second instar larvae of *Aedes aegypti* [19]. It, thus, seems desirable to assay the butenolactones **1**–**6** for the same biological properties.

In addition, bioactivity screenings revealed that rotundifolide A (**1**) exhibited significant *in vitro* inhibitory activity ($IC_{50} = 9.2\,\mu M$) against the enzyme protein tyrosine phosphatase-1B (PTP1B), a tyrosine phosphatase that has been implicated as a key target for type-II diabetes [20].

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Experimental Part

General. Column chromatography (CC): commercial silica gel (*Qing Dao Hai Yang Chemical Group Co.*; 100–200 and 200–300 mesh). Anal. TLC: precoated silica gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60 F-254*). Reversed-phase HPLC: *Agilent 1100* liquid chromatograph, equipped with an *Agilent 1100-G1314A* variable-wavelength detector; semi-prep. *Develosil ODS-5* column (5 μm; 9.6 mm (i.d.) × 25 cm); t_R in min. Optical rotation: *Perkin-Elmer 341* polarimeter. UV Spectra: *Varian-Cary 300-Bio* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna-FT-IR-750* spectrometer; ν_{max} in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker DRX-400* spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C; chemical shifts δ in ppm, with residual $CHCl_3$ (δ(H) 7.26, δ(C) 77.0) as internal standard, coupling constants *J* in Hz; assignments supported by ^1H,^1H-COSY, HMQC, and HMBC experiments. EI-MS and HR-EI-MS: *MAT-95* mass spectrometer; in *m*/*z* (rel. %). ESI-MS and HR-ESI-MS: *Q-TOF-Micro* LC-MS-MS mass spectrometer; in *m*/*z*.

Plant Material. The sample examined was collected from the Guangdong Province of China in August 2001 and identified by Prof. *F.-W. Xin* of the South China Institute of Botany, Chinese Academy of Sciences. A voucher specimen (no. PL02-5) is deposited in the Herbarium of the Institute of Materia Medica, SIBS-CAS.

Extraction and Purification. The powdered bark of *L. rotundifolia* var. *oblongifolia* (1 kg) was repeatedly extracted with MeOH (9 l) at r.t. The extract was evaporated to give a brown syrup (132 g), which was partitioned with solvents into AcOEt-soluble (65 g) and BuOH-soluble (20 g) fractions, respectively. The AcOEt-soluble portion was subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): *Fractions 1–15*. *Fr. 5* (110.3 mg) was subjected to CC (silica gel, petroleum ether/Et_2O gradient): mixture of lactones (*Fr. 5.1*; 20.6 mg), crude **1** (*Fr. 5.2*; 15.2 mg), and crude **2** (*Fr. 5.3*; 10.6 mg). *Fr. 5.1* was further separated by prep. HPLC (MeOH/H_2O 8:2, flow rate 2 ml/min): **3** (4.6 mg; t_R 20.9), **5** (3.2 mg; t_R 21.8), **6** (2.8 mg; t_R 22.5), and **4** (1.8 mg; t_R 31.7). The crude **1** and **2** were each resubjected to CC (silica gel, petroleum ether/Et_2O 4:6): pure **1** (10.0 mg) and **2** (8.2 mg).

Rotundifolide A (=*(5*S*)-3-(Dodec-11-enyl)-4-hydroxy-5-methylfuran-2(5*H*)-one*; **1**): Colorless oil. $[\alpha]_D = +11$ ($c = 0.25$, dioxane). UV (EtOH): 210 (4.2). IR (KBr): 3399, 2923, 2850, 1747, 1660, 1641, 1072, 912. ^1H- and ^{13}C-NMR: *Table*. ESI-MS: 279 ($[M-H]^-$). EI-MS: 280 (15, M^+), 262 (8), 128 (92), 115 (100), 98 (28), 69 (32), 55 (56). HR-EI-MS: 280.2042 ($C_{17}H_{28}O_3^+$; calc. 280.2038).

Rotundifolide B (=*(5*S*)-3-(Dodec-11-ynyl)-4-hydroxy-5-methylfuran-2(5*H*)-one*; **2**): Colorless oil. $[\alpha]_D = +21.7$ ($c = 0.20$, dioxane). UV (EtOH): 210 (4.2). IR (KBr): 3309, 2923, 2850, 2118, 1757, 1660, 1465, 1071, 721, 636. ^1H- and ^{13}C-NMR: *Table*. ESI-MS: 277 ($[M-H]^-$).

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