DOI 10.5012/bkcs.2011.32.7.2443

Notes

New Cytotoxic δ-Valerolactones from *Cornus walteri*

Ki Hyun Kim, Young June Shin, Sang Un Choi,† and Kang Ro Lee*

Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea
**E-mail: krlee@skku.ac.kr*
†Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea
Received April 20, 2011, Accepted May 23, 2011

Key Words : *Cornus walteri*, Cornaceae, δ-Valerolactone, Cytotoxicity

Cornus walteri Wanger (Cornaceae) is a deciduous shrub distributed in valley areas of Asia.[1] Its fruits and leaves have been used in Chinese folk medicine for treatment of inflammation of the skin or boils caused by lacquer poison,[2] and Koreans have used its leaves as an antidiarrheal.[3] The presence of metabolites including gallic acid and flavonoids from previous investigations on *C. walteri* was confirmed.[2,3] Extracts of *C. walteri* were found to inhibit NO production in lipopolysaccharide (LPS)-activated macrophages.[4] Extracts also inhibited elastase and tyrosinase, and had *anti*-hyperglycemic, and *anti*-obesity effects.[5,6] In our search for bioactive constituents from Korean medicinal plants, we found that a methanol extract of stems and stem bark of *C. walteri* had considerable cytotoxicity against some cancer cell lines,[7] and recently reported the identification of triterpenoids with cytotoxicity from the *n*-hexane-soluble fraction of the extract.[7] In our continuing efforts to study the active constituents of the MeOH extract, six δ-valerolactones (**1-6**) were isolated from the $CHCl_3$-soluble fraction of the MeOH extract using bioassay-guided fractionation (Figure 1). Two of these compounds (**1-2**) are new, while two others (**3-4**) have been previously reported but only as synthetic products. We report herein the isolation, structural elucidation (**1-4**), and cytotoxicity of all of the isolated compounds.

Compound **1** was obtained as a colorless gum. The molecular formula was established as $C_6H_8O_3$ (3 degrees of unsaturation) evidenced from the $[M+H]^+$ peak at *m/z* 129.0550 (calcd. for $C_6H_9O_3$: 129.0552) in the HR-ESIMS. In compliance with the formula, the presence of hydroxyl and ester carbonyl groups in the molecule could be proposed from the IR absorption bands of **1** at 3362 and 1703 cm^{-1}. The ^1H NMR spectrum of **1** (Table 1) indicated the presence of an olefinic proton at δ_H 5.77 (1H, s, H-3), an oxygenated methine at δ_H 4.15 (1H, dd, J = 4.5, 4.0 Hz, H-5), and a methyl group at δ_H 2.06 (3H, s, H-7). The ^1H NMR signals for an oxygenated methylene group at δ_H 4.37 (1H, dd, J = 12.0, 4.0 Hz, H-6a) and 4.33 (1H, dd, J = 12.0, 4.5 Hz, H-6b)

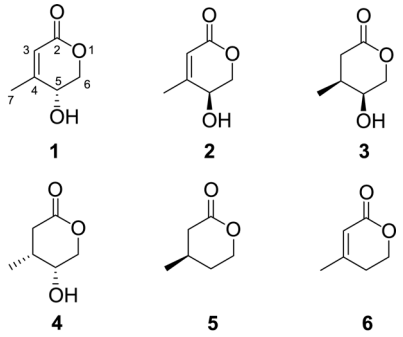

Figure 1. Chemical structures of compounds **1-6**.

Table 1. ^1H NMR and ^{13}C NMR spectral data of compounds **1-4**[a]

H/C	1		2		3		4	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
2		164.3		164.0		176.6		176.5
3	5.77 s	117.3	5.80 s	117.6	2.73 dd (17.5, 9.0) 2.22 dd (17.5, 9.0)	37.2	2.75 dd (17.5, 9.0) 2.24 dd (17.5, 9.0)	37.2
4		158.9		158.4	2.51 m	31.3	2.52 m	31.3
5	4.15 dd (4.5, 4.0)	64.6	4.16 dd (4.5, 4.0)	64.8	4.13 m	87.4	4.13 m	87.3
6	4.37 dd (12.0, 4.0) 4.33 dd (12.0, 4.5)	71.7	4.39 dd (12.0, 4.0) 4.36 dd (12.0, 4.5)	71.6	3.89 dd (12.5, 2.5) 3.66 dd (12.5, 4.5)	62.8	3.92 dd (12.5, 2.5) 3.69 dd (12.5, 4.5)	62.8
7	2.06 s	20.1	2.07 s	20.1	1.16 d (6.5)	17.9	1.18 d (6.5)	17.9

[a] ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in $CDCl_3$. Chemical shifts are given in δ values. Proton coupling constants (J) in Hz are given in parentheses.

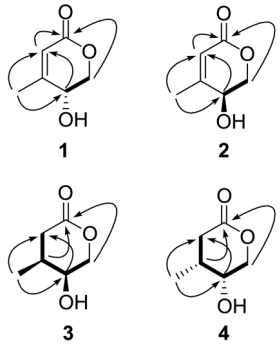

Figure 2. Key ^{1}H-^{1}H COSY (---) and HMBC (→) correlations of **1-4**.

were also observed and the ^{1}H-^{1}H COSY correlation from H-5 to H_2-6 indicated the presence of the fragment (-CH-CH_2-). Furthermore, the co-occurrence of a double bond at C-3/4 and a methyl group at C-4 was deduced from the HMBC experiment showing correlations of H-7 (δ_H 2.06)/C-3 (δ_C 117.3) and C-5 (δ_C 64.6) and correlation of H-5 (δ_H 4.15)/C-3 (δ_C 117.3) (Figure 2). The chemical shift of C-2 (δ_C 164.3) indicating an ester carbonyl group and the unsaturation index suggested the skeleton of **1** to be δ-valerolactone. That hypothesis was reinforced by the HMBC correlation of H-3 (δ_H 5.77) with C-2 (δ_C 164.3) and the correlation of H-6 (δ_H 4.37 and 4.33) with C-2 (δ_C 164.3), which finally led to the establishment of the planar structure for **1** (Figure 2). The absolute configuration of C-5 was determined using a modified Mosher's method.[7] Treatment of **1** with (*R*)- and (*S*)-MTPA-Cl gave the (*S*)- and (*R*)-MTPA esters **1s** and **1r**, respectively. The ^{1}H NMR signals of the two MTPA esters were assigned on the basis of their ^{1}H-^{1}H COSY spectra, and the Δδ values ($\delta_S - \delta_R$) were then calculated (Figure 3, see the Supporting Information). The results indicated that the absolute configuration of C-5 was *R*. Thus, compound **1** was assigned as (*R*)-5,6-dihydro-5-hydroxy-4-methyl-2*H*-pyran-2-one (walterolactone A).

Compound **2** was also obtained as a colorless gum with the molecular formula $C_6H_8O_3$ based on the $[M + H]^+$ peak at *m/z* 129.0557 (calcd. for $C_6H_9O_3$: 129.0552) in the HR-ESIMS. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were almost identical to those of **1**. The planar structure of **2** was confirmed by a set of 2D NMR experiments (Figure 2) that led to the exact assignment of all ^{1}H and ^{13}C NMR signals of

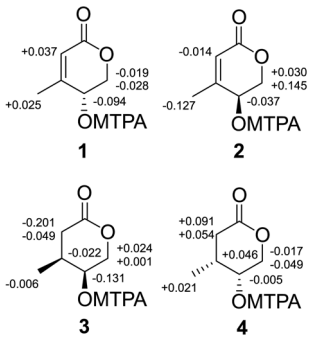

Figure 3. Δδ Values ($\delta_S - \delta_R$) in ppm of the two MTPA esters derived from **1-4**.

2 (Table 1). However, the optical rotation of **2** ($[\alpha]_D^{25}$: +28.3) was almost of the same value but of opposite sign to that of **1** ($[\alpha]_D^{25}$: −31.5), which suggested that compound **2** could be an enantiomer of **1**. Similarly as described for **1**, the absolute configuration of C-5 in **2** was determined using a modified Mosher's method,[7] which proved the *S*-configuration for C-5 (Figure 3, see the Supporting Information). Thus, compound **2** was characterized as (*S*)-5,6-dihydro-5-hydroxy-4-methyl-2*H*-pyran-2-one (walterolactone B).

Compounds **3** ($C_6H_{10}O_3$) and **4** ($C_6H_{10}O_3$) were isolated as colorless gums showing an $[M + H]^+$ peak at *m/z* 131.0711 and 131.0713 (calcd. for $C_6H_{11}O_3$: 131.0708) in the HR-ESI-MS, respectively. Their NMR spectral data (Table 1) were almost identical to each other, and displayed the presence of one ester carbonyl, one methyl, two methylenes, one methine, and one hydroxylated methine, which were in agreement with those of *cis*-β-methyl-γ-hydroxy-δ-valerolactone.[8] The planar structures of **3** and **4** were confirmed by 2D NMR experiments (Figure 2). The COSY spectrum revealed a spin system composed of H-3/H-4/H-5/H-6, and HMBC correlations of H-7/C-3, H-7/C-5, H-5/C-3, H-4/C-2, and H-6/C-2 were shown in their HMBC spectrum (Figure 2), indicative of the presence of a methyl group at C-4 and an ester carbonyl group at C-2. The *cis*-configuration between the methyl group at C-4 and the hydroxyl group at C-5 of **3** and **4** was assigned by the chemical shift (δ_H 4.13 for **3**; δ_H 4.13 for **4**) of H-5, which was in agreement with that of *cis*-β-methyl-γ-hydroxy-δ-valerolactone (δ_H 4.12-4.18 for *cis*-form; δ_H 4.50-4.55 for *trans*-form).[8] Finally, their absolute configuration of C-5 was determined using the modified Mosher's method as described for **1**,[7] which proved the *S*-configuration for C-5 of **3** and the *R*-configuration for C-5 of **4** (Figure 3, see the Supporting Information). Therefore, the structures of **3** and **4** were elucidated as (4*S*,5*S*)-tetrahydro-5-hydroxy-4-methyl-2*H*-pyran-2-one (walterolactone C) and (4*R*,5*R*)-tetrahydro-5-hydroxy-4-methyl-2*H*-pyran-2-one (walterolactone D), respectively.

Known compounds were identified as (*R*)-4-methyltetrahydro-2*H*-pyran-2-one (**5**)[9] and 4-methyl-5,6-dihydro-2-pyranone (**6**)[10] by comparison of physicochemical and spectroscopic data with previously reported literature values.

Table 2. Cytotoxicity of compounds **1-6** against four cultured human cancer cell lines using an SRB bioassay *in vitro*

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	>30.0	>30.0	23.94	>30.0
2	>30.0	>30.0	21.10	>30.0
3	27.23	>30.0	28.43	>30.0
4	27.89	>30.0	22.68	>30.0
5	>30.0	>30.0	23.38	>30.0
6	28.45	5.76	4.75	28.12
Doxorubicin[b]	0.007	0.006	0.001	0.182

[a]IC_{50} value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*. [b]Doxorubicin as a positive control.

To the best of our knowledge, this is the first study to report the presence of δ-valerolactones in the genus *Cornus*.

In this study, the cytotoxicities of **1-6** against the A549, SK-OV-3, SK-MEL-2, and HCT15 human cancer cell lines were evaluated using the Sulforhodamine B (SRB) bioassay *in vitro*.[11] The results of this bioassay (Table 2) showed that all the tested δ-valerolactones (**1-6**) exhibited consistent cytotoxicity against the SK-MEL-2 cell line with IC_{50} values in the range of 4.75-28.43 μM. In particular, compound **6** showed significant cytotoxicity against all of the cell lines tested with IC_{50} values of 28.45, 5.76, 4.75, and 28.12 μM, respectively, for the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines. These bioactivity data suggest that these compounds may be good bioactive molecules for the treatment of various cancers.

Experimental Section

Plant Materials. Stems and stem bark of *C. walteri* were collected on Jeju Island, Korea, in October, 2005, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2005-10a) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. Stems and stem bark of *C. walteri* (2.5 kg) were dried, chopped, and extracted with 80% MeOH (2 × 6 h) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a MeOH extract (220 g), which was suspended in distilled H_2O (7.2 L) and then successively partitioned with *n*-hexane, $CHCl_3$, and *n*-BuOH, yielding 9.5, 25.0, and 43.0 g of residue, respectively. Each fraction was evaluated for cytotoxicity against human tumor cell lines, the A549, SK-OV-3, and SK-MEL-2 cell lines, using a SRB bioassay. We selected the $CHCl_3$-soluble fraction for current phytochemical investigation, since the $CHCl_3$-soluble fraction showed significant cytotoxic activity against tested tumor cell lines. Purification of six δ-valerolactones (**1-6**) was described in the Supporting Information.

Walterolactone A (1): Colorless gum, $[\alpha]_D^{25}$: −31.5 (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 219 (3.0) nm; IR (KBr): ν_{max} = 3362, 2947, 2833, 1703, 1452, 1032, 671 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESI-MS: *m/z* = 129 $[M + H]^+$; HR-ESI-MS: *m/z* = 129.0550 $[M + H]^+$ (calcd. for $C_6H_9O_3$: 129.0552).

Walterolactone B (2): Colorless gum, $[\alpha]_D^{25}$: +28.3 (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 219 (3.2) nm; IR (KBr): ν_{max} = 3362, 2947, 2833, 1703, 1453, 1033, 671 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESI-MS: *m/z* = 129 $[M + H]^+$; HR-ESI-MS: *m/z* = 129.0557 $[M + H]^+$ (calcd. for $C_6H_9O_3$: 129.0552).

Walterolactone C (3): Colorless gum, $[\alpha]_D^{25}$: +36.4 (*c* 0.55, $CHCl_3$); IR (KBr): ν_{max} = 3375, 2946, 2833, 1759, 1452, 1032, 671 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESI-MS: *m/z* = 131 $[M + H]^+$; HR-ESI-MS: *m/z* = 131.0711 $[M + H]^+$ (calcd. for $C_6H_{11}O_3$: 131.0708).

Walterolactone D (4): Colorless gum, $[\alpha]_D^{25}$: −41.0 (*c* 0.10, $CHCl_3$); IR (KBr): ν_{max} = 3356, 2947, 2833, 1758, 1452, 1032, 671 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; ESI-MS: *m/z* = 131 $[M + H]^+$; HR-ESI-MS: *m/z* = 131.0713 $[M + H]^+$ (calcd. for $C_6H_{11}O_3$: 131.0708).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 1-4. To a stirred solution of **1** (6.0 mg) in pyridine (400 μL) was added 4-(dimethylamino)pyridine (3 mg) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, 10 μL). The mixture was stirred at room temperature for 16 h. The reaction mixture was then passed through a silica gel Waters Sep-Pak Vac 6cc and eluted with *n*-hexane-EtOAc (1:1) to give the respective (*R*)-Mosher ester **1r**. Treatment of **1** (6.0 mg) with (*R*)-MTPA-Cl (10 μL) as described above yielded the corresponding (*S*)-MTPA ester **1s**. Similarly, treatment of **2-4** with (*S*)- and (*R*)-MTPA-Cl afforded the respective Mosher esters **2r**, **2s**, **3r**, **3s**, **4r**, and **4s**.

A detailed description of the bioassays is available as the Supporting Information. The positive control, doxorubicin (purity ≥ 98%) was purchased from the Sigma Corporation.

Acknowledgments. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20100029358). We thank Drs. E. J. Bang, S. G. Kim, and J. J. Seo at the Korea Basic Science Institute for their aid in obtaining the NMR and mass spectra.

Supporting Information. The spectral data of compounds **1-4**, the general experimental procedures, the isolation details, ^1H NMR data of the (*S*)- and (*R*)-MTPA esters of **1-4**, and bioassays protocols are available on request from the correspondence author.

References

1. Lee, T. B. *Coloured Flora of Korea*, Hyangmoonsa: Seoul, 2006; p 851.
2. Choi, W. H.; Park, W. Y.; Hwang, B. Y.; Oh, G. J.; Kang, S. J.; Lee, K. S.; Ro, J. S. *Kor. J. Pharmacogn.* **1998**, *29*, 217.
3. Yook, C. S. *Coloured Medicinal Plants of Korea*; Academybook: Seoul, 1993; p 368.
4. Yang, E. J.; Yim, E. Y.; Song, G.; Kim, G. O.; Hyun, C. G. *Interdisc. Toxicol.* **2009**, *2*, 245.
5. Moon, J. Y.; Yim, E. Y.; Song, G.; Lee, N. H.; Hyun, C. G. *EurAsia J. BioSci.* **2010**, *4*, 41.
6. Lim, C. S.; Li, C. Y.; Kim, Y. M.; Lee, W. Y.; Rhee, H. I. *J. Korean. Soc. Appl. Biol. Chem.* **2005**, *48*, 103.
7. Kim, K. H.; Choi, S. U.; Kim, Y. C.; Lee, K. R. *J. Nat. Prod.* **2011**, *74*, 54.
8. Shibata, I.; Matsuo, F.; Baba, A; Matsuda, H. *J. Org. Chem.* **1991**, *56*, 475.
9. Brown, M. K.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2008**, *130*, 12904.
10. Shimomura, H.; Sashida, Y.; Mimaki, Y.; Adachi, T.; Yoshinari, K. *Chem. Pharm. Bull.* **1989**, *37*, 829.
11. Skehan, P.; Stroreng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.