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New γ-Lactone Carboxylic Acids from the Lichen *Parmotrema praesorediosum* (Nyl.) Hale, Parmeliaceae

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Abstract: From the lichen *Parmotrema praesorediosum* (Nyl.) Hale, Parmeliaceae, collected in Vietnam, five new (**1**, **2**, **3**, **5**, **6**) and one known (**4**) γ-lactonic acids were isolated. The structures of the new compounds were established by 1D and 2D NMR spectroscopy, as well as high resolution-electrospray ionization (HR-ESI)-MS analysis. The absolute configuration of new compounds was determined by ultraviolet circular dichroism spectroscopy. Compounds were evaluated for their cytotoxic activity against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer) and MCF-7 (human breast cancer) cell lines at the concentration of 100 μg/mL. Among six compounds, **1** possessed medium activity against MCF-7 cell line with the inhibition of 52.5%. Meanwhile, the rest showed no cytotoxic activity against three surveyed cancer cell lines.

Keywords: *Parmotrema praesorediosum*; Parmeliaceae; lichen; aliphatic acids. © 2015 ACG Publications. All rights reserved.

1. Introduction

5-Oxo-tetrahydro-3-furancarboxylic acid derivatives, or paraconic acids, which constitute a small class of trisubstituted γ–butyrolactones, are secondary metabolites of mosses, fungi and lichens [1-4]. This family comprises an alkyl chain, a carboxyl group and a methyl or methylene group at C-2, C-3, and C-4, respectively, in the γ–butyrolactone skeleton. A double bond between C-3 and C-4 is

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often encountered. Some of γ–lactonic acids are biologically significant. For example, (+)-nephrosteranic acid [5], methylenolactocin [6], and (-)-protolichesterinic acid [7] showed anti-tumor and anti-fungal properties. The bioactivities were ascribed to the presence of either a methyl or a methylene group at the α-position of the γ–butyrolactones, which plays an important role in determining the physiological properties of compounds [6,7]. Previous phytochemical studies on *Parmotrema praesorediosum* (Nyl.) Hale, collected on a betel nut tree, in southern Thailand, revealed that this species contained some lactone fatty acids [8], prompting us to further investigate this plant species to search for biologically active compounds. This paper describes the isolation, structure elucidation of five new and one known γ-lactonic acids from *P. praesorediosum*.

All of isolated compounds did possess a methyl group at C-4 and a double bond between C-3 and C-4. Compounds were evaluated for their cytotoxic activity against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer) and MCF-7 (human breast cancer) cell lines at the concentration of 100 μg/mL.

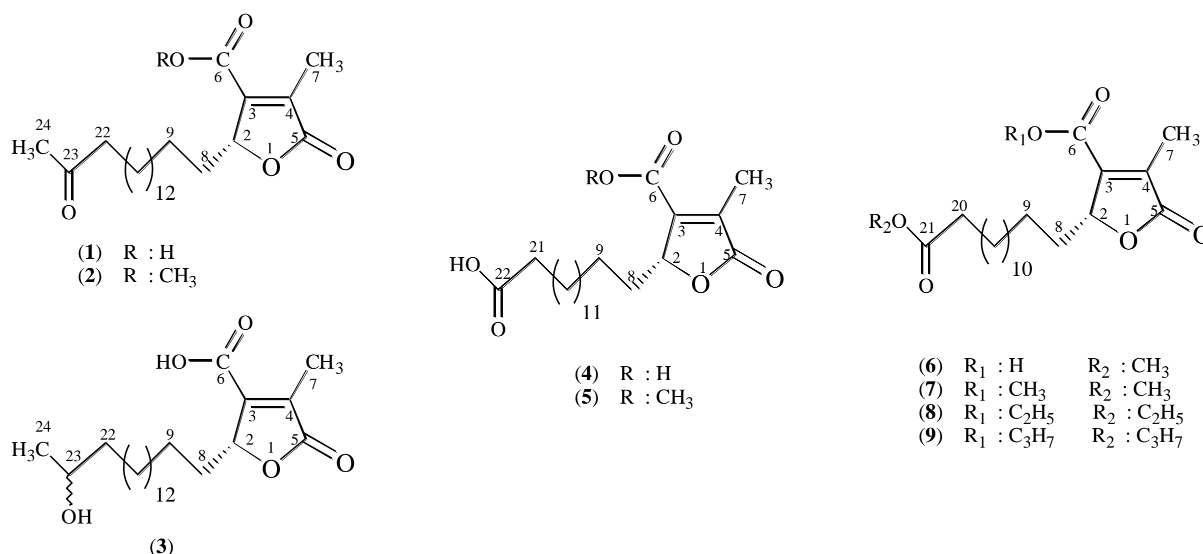

Figure 1. Chemical structures of compounds **1–9**

2. Materials and Methods

2.1. General experimental procedures

NMR spectra were recorded on a Bruker Avance III spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR, using residual solvent signal as internal reference: chloroform-*d* δ_H 7.24, δ_C 77.23 and acetone-d_6 δ_H 2.09, δ_C 206.31, 30.6. The HR–ESI–MS were recorded on a MicroOTOF–Q mass spectrometer. Melting points were determined on Maquenne block and were uncorrected. Optical rotations (in MeOH) were measured on a Kruss digital polarimeter. Absorption and CD spectra (in chromatographic grade MeOH, 0.5 cm optical cell length) were measured on a JASCO V-570 spectrophotometer and on a JASCO J-820E spectropolarimeter, respectively. TLC was carried out on precoated silica gel 60 F254 (Merck). Spots were detected under ultraviolet (UV) irradiation or visualized by spraying with 10% aqueous H_2SO_4 followed by heating. Gravity column chromatography was performed with silica gel 60 (0.040 – 0.063 mm, Himedia).

2.2. Plant material

The material was collected on the bark of *Dipterocarpus alatus* at Nam Cat Tien National Forest Reserve and Intermediate zones Nam Cat Tien village, Tan Phu district, Dong Nai province, Vietnam in June 2009. The botanical species of *Parmotrema praesorediosum* (Nyl.) Hale was authenticated by Dr. Harrie Sipman, from Freie University of Berlin, Germany. A voucher specimen (No US B020) was deposited at the Herbarium of the Department of Organic Chemistry, Faculty of Chemistry, University of Science, National University - Ho Chi Minh City-Vietnam.

2.3. Extraction and Isolation

After carefully removing by hand the exotic bark out of the lichen thalli, the fresh material (5.0 kg) was cleaned under running tap water and air-dried. The ground powder sample (3.0 kg) was macerated with ethanol (8 L x 4) at room temperature and after filtration the ethanol solution was concentrated under reduced pressure to yield a residue of 450 g. This crude lichen extract was subjected to silica gel solid phase extraction then successively eluted with solvents of increasing polarity to give corresponding extracts: light petroleum ether (40.0 g), chloroform (105.0 g), ethyl acetate (50.1 g), acetone (45.2 g) and methanol (36.9 g).

The petroleum ether extract was subjected to silica gel CC eluting with a solvent system of petroleum ether–EtOAc (from 9:1 to 1:1, with an increase of 10% of EtOAc in each step) to afford fractions E1 to E9. Fraction E5 (4.5 g) was subjected to silica gel column chromatography eluted with petroleum ether –EtOAc (9:1) to give **2** (25 mg). The same method was applied to fraction E7 (2.5 g) to afford **5** (250 mg).

The chloroform extract (105.0 g) was applied to silica gel CC and eluted with petroleum ether–EtOAc (10:0 to 0:10 with increasing EtOAc) to yield twenty three fractions from C1 to C23. Fraction C13 (5.7 g) was applied on silica gel column and eluted with a gradient solvent system of petroleum ether–$CHCl_3$ (8:2) to give **6** (1.0 g). Fraction C15 (3.4 g) was rechromatographed, eluted with petroleum ether–$CHCl_3$ (8:2) to give **1** (200 mg). Fraction C16 (4.2 g) was applied on silica gel column and eluted with a gradient solvent system of petroleum ether–$CHCl_3$ (5:5) to give **4** (295 mg). Fraction C17 (6.1 g) was rechromatographed, eluted with $CHCl_3$–MeOH (95:5) to give **3** (15 mg).

(+)-Vinapraesorediosic acid A (**1**): White needles, mp. 104–105 ^oC ($CHCl_3$), $[\alpha]_D^{23}$ + 518 (*c* = 0.006, EtOH). CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 221 (–8.7). IR (KBr) ν_{max} cm^{-1}: 3444 (O–H), 1740 (C=O, lactone), 1706 (C=O, ester), 1217 (C–O). HR-ESI-MS *m/z* 395.2781 $[M+H]^+$ (calcd. for $C_{23}H_{38}O_5$+H: 395.2797). ^1H NMR ($CDCl_3$) data see Table 1 and ^{13}C NMR ($CDCl_3$) data see Table 2.

(+)-6-Methyl vinapraesorediosate A (**2**): White needles, mp. 89–90 ^oC ($CHCl_3$). $[\alpha]_D^{23}$ + 837 (*c* = 0.006, EtOH). CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 225 (–6.4). IR (KBr) ν_{max} cm^{-1}: 3440 (O–H), 1767 (C=O, lactone), 1706 (C=O, ester). HR-ESI-MS *m/z* 409.2988 $[M+H]^+$ (calcd. for $C_{24}H_{40}O_5$+H: 409.2954). ^1H NMR ($CDCl_3$) data see Table 1 and ^{13}C NMR ($CDCl_3$) data see Table 2.

(+)-Vinapraesorediosic acid B (**3**): White needles, mp. 124–125 ^oC ($CHCl_3$). $[\alpha]_D^{23}$ + 22 (*c* = 0.0013, $CHCl_3$). CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 220 (–7.1). HR-ESI-MS *m/z* 419.2754 $[M+Na]^+$ (calcd. for $C_{23}H_{40}O_5$+Na: 419.2773). ^1H NMR (acetone-d_6) data see Table 1 and ^{13}C NMR (acetone-d_6) data see Table 2.

(+)-Praesorediosic acid (**4**): White needles, mp. 139–140 ^oC ($CHCl_3$). $[\alpha]_D^{23}$ + 513 (*c* = 0.001, MeOH). CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 223 (–11.1). HR-ESI-MS *m/z* 405.2238 $[M+Na]^+$ (calcd. for $C_{21}H_{34}O_6$+Na: 405.2253). ^1H NMR (acetone-d_6) data see Table 1 and ^{13}C NMR (acetone-d_6) data see Table 2.

(+)-6-Methyl praesorediosate (**5**): White needles, mp. 102–103 ^oC ($CHCl_3$). $[\alpha]_D^{23}$ + 450 (*c* = 0.001, EtOH). CD (MeOH) λ_{max} nm ($\Delta\varepsilon$) 226 (–8.9). IR (KBr) ν_{max} cm^{-1}: 3144 (O–H), 1735 (C=O,

lactone), 1724 (C=O, ester). HR-ESI-MS *m/z* 397.2596 $[M+H]^+$ (calcd. for $C_{22}H_{36}O_6$+H: 397.2590). ^1H NMR ($CDCl_3$) data see Table 1 and ^{13}C NMR ($CDCl_3$) data see Table 2.

(+)-Vinapraesorediosic acid C (**6**): White needles, mp. 132–133 °C ($CHCl_3$). $[\alpha]_D^{23}$ + 130 (*c* = 0.001, EtOH). CD (MeOH) λ_{max} nm (Δε) 223 (–8.7). IR (KBr) ν_{max} cm^{-1}: 3423 (O–H), 1739 (C=O, lactone), 1700 (C=O, ester). HR-ESI-MS *m/z* 405.2200 $[M+Na]^+$ (calcd. for $C_{21}H_{34}O_6$+Na: 405.2253). ^1H NMR ($CDCl_3$) data see Table 1 and ^{13}C NMR ($CDCl_3$) data see Table 2.

Methylation of ***6***: 20 mg of **6** in 10 mL methanol which was acidified with H_2SO_4 98% to get pH = 0-1 was stirred for 8 hours at a temperature of 80 °C. The reaction mixture was concentrated in the reduced pressure and the residue was purified by preparative TLC (petroleum ether–$CHCl_3$, 5:5) to yield **7,** a transparent oil (4.0 mg), **7**: CD (MeOH) λ_{max} nm (Δε) 222 (–7.8). ^1H NMR ($CDCl_3$) data see Table 1 and ^{13}C NMR ($CDCl_3$) data see Table 2.

Ethylation of ***6***: 20 mg of **6** in 5 mL ethanol which was acidified with H_2SO_4 98% to get pH = 0-1 was stirred for 8 hours at a temperature of 80 °C. The reaction mixture was concentrated under reduced pressure and the residue was purified by preparative TLC (petroleum ether–$CHCl_3$, 5:5) to yield **8,** a transparent oil (6.0 mg). **8**: CD (MeOH) λ_{max} nm (Δε) 228 (–6.7). ^1H NMR ($CDCl_3$) δ: 5.10 (1H, m), 4.34 (2H, m), 4.12 (2H, q, *J* =7.0 Hz), 2.29 (2H, m), 2.18 (3H, d, *J* =2.0 Hz), 2.07 (1H, m), 1.58 (1H, m), 1.37 (6H, t, *J*=7.0 Hz), and 1.23–1.26 (-CH_2-, br s); ^{13}C NMR ($CDCl_3$) δ: 174.0 (C-20), 173.1 (C-1), 162.4 (C-6), 148.1 (C-3), 137.3 (C-2), 81.5 (C-4), 61.7, 14.4 (20-OC_2H_5), 60.3, 14.3 (6-OC_2H_5), 34.3 (C-19), 32.9 (C-7), 29.7–29.3 (-CH_2-), 25.2 (C-18), 24.8 (C-8), and 10.9 (C-5).

n-Propylation of **6**: 30 mg of **6** in 10 ml *n*-propanol which was acidified with H_2SO_4 98% to get pH = 0-1 was stirred for 9 hours at a temperature of 80 °C. The reaction mixture was concentrated under reduced pressure and the residue was purified by preparative TLC (petroleum ether–$CHCl_3$, 5:5) to yield **9,** a transparent oil (8.2 mg). **9**: CD (MeOH) λ_{max} nm (Δε) 225 (–11.5). ^1H NMR ($CDCl_3$) δ: 5.10 (1H, m), 4.26 (2H, m), 4.02 (2H, t, J =6.5 Hz), 2.29 (2H, t, J =7.5 Hz), 2.19 (3H, d, J =2.0 Hz), 2.08 (1H, m), 1.76 (2H, sext, J=7.0 Hz), 1.24 (-CH_2-, br s), 1.01 (3H, t, J =7.5 Hz), and 0.94 (3H, t, J =7.5 Hz); ^{13}C NMR ($CDCl_3$) δ: 174.1 (C-20), 173.1 (C-1), 162.5 (C-6), 148.2 (C-3), 137.3 (C-2), 81.6 (C-4), 67.3, 22.2, 11.0 (20-OC_3H_7), 66.0, 22.1, 10.6 (6-OC_3H_7), 34.6 (C-19), 33.0 (C-7), 29.8–29.3 (-CH_2-), 25.2 (C-18), 24.9 (C-8), and 10.5 (C-5).

2.4. TD-DFT Calculations of the CD spectra

The structures of the model molecule were optimized at the level of B3LYP/6-311++G** in vacuo and in CPCM solvent model of methanol. The populations of the two stable conformers were calculated based on the relative energies with the Boltzmann distribution at 300 K. The optimization under the CPCM solvent model of methanol did not change these geometries or populations significantly. The electronic CD spectra of the stable conformers were calculated at the TD-DFT theory with the same basis sets as the optimizations by using Gaussian09 program, fitted by Gaussian curves with 0.30 eV line width, and then weighted-averaged based on the Boltzamann population.

2.5. Biological Assays

Determination of cytotoxic activities against the HeLa (human epithelial carcinoma), MCF–7 (human breast cancer) and NCI-H460 (human lung cancer) cell lines of tested samples was performed at the concentration of 100 μg/mL using the Sulforhodamine B (SRB) assay [12] with camptothecin as the positive control. All cells were cultured in E'MEM medium (Eagle's Minimal Essential Medium) supplemented with 10% foetal bovine serum (FBS), 1% of 2 mM L-glutamine, 50 IU/mL penicillin, 50 μg/mL streptomycin and maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity. Viable cells were counted and inoculated in 96-well plate with density of 104 cells/100 μL/well. After 24 h the cells were treated with pure compound while the control wells were added only by 100 μL medium. All experiments were in triplicate. The plates were incubated in an atmosphere of 5% CO_2,

95% humidity at 37 °C for 48 h. Adherent cell cultures were fixed by adding 50 μL of cold 50% (w/v) trichloroacetic acid per well and incubated at 4 °C for 1 h. The plates were washed five times with distilled water and air dried. Then a solution of 50 μL of SRB (0.4% *w/v* in 1% acetic acid) was added to each well and the plate was stained at room temperature for 30 min. The SRB solution was removed out of plates by rinsing 4 times with a 1% glacial acetic acid solution (200 μL/well). The plates were air-dried for 12–24 h. The bound SRB was dissolved to each well by adding 100 μL of 10 mM Tris Base (pH 10.5). The plates were shaken gently for 20 min and the optical density of each well was read using a scanning multiwall spectrophotometer at a test wavelength of 492 nm and a reference wavelength of 620 nm. The optical density (OD) of SRB in each well is directly proportional to the cell number. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells)

3. Results and Discussion

The thallus of *Parmotrema praesorediosum* (Nyl.) Hale was collected at Dong Nai Province, Vietnam. By using silica gel column chromatography with petroleum ether and chloroform as mobile phases, six compounds (**1–6**) were isolated from the plant. The positive-ion high-resolution-electrospray ionization mass spectrum (HR-ESI-MS) confirmed the molecular formulas of these compounds were $C_{23}H_{38}O_5$, $C_{24}H_{40}O_5$, $C_{23}H_{40}O_5$, $C_{21}H_{34}O_6$, $C_{22}H_{36}O_6$ and $C_{21}H_{34}O_6$, respectively. Their IR, CD and NMR spectra suggested that they possessed close chemical structure relating to the (+)-praesorediosic acid [8].

Compound **1** was obtained as white needles. The HR-ESI-MS showed the quasi–molecular ion peak at *m/z* 395.2781 $[M+H]^+$ corresponding to the molecular formula $C_{23}H_{38}O_5$ which implied five degrees of unsaturation. Its IR spectrum displayed two intense absorptions at 1740 cm^{-1} and 1706 cm^{-1} that were assigned to the lactone ring and the carboxyl group, respectively.

Table 1.^{1}H NMR data of compounds **1–7** (δ in ppm, J in Hz).

No	1[a]		2[a]		3[b]		4[b]		5[a]		6[a]		7[a]	
2	5.12	m	5.09	m	5.14	m	5.14	m	5.09	m	5.12	m	5.10	m
6-OCH$_3$	–		3.89	s	–		–		3.88	s	–		3.89	s
7	2.23	d (2.0)	2.18	d (2.0)	2.12	d (2.0)	2.12	d (2.0)	2.18	d (2.5)	2.24	d (2.0)	2.19	d (2.0)
8	2.11	m	2.07	m	1.62	m	2.05	m	2.05	m	2.14	m	2.07	m
	1.58	m	1.56	m	1.39	m	1.35	m	1.55	m	1.60	m	1.55	m
(-CH$_2$-)$_n$	1.25–1.28	br s	1.25–1.27	br s	1.29	br s	1.29–1.38	br s	1.23–1.30	br s	1.25	br s	1.24	br s
20	1.25–1.28	br s	1.25–1.27	br s	1.29	br s	1.60	m	1.64	m	2.32	t (7.5)	2.30	t (7.5)
21	1.25–1.28	br s	1.25–1.27	br s	1.29	br s	2.27	t (7.5)	2.34	t (7.5)	–		–	
21-OCH$_3$	–		–		–		–		–		3.68	s	3.66	s
22	2.43	t (7.5)	2.41	t (7.5)	2.05	m	–		–		–		–	
23	–		–		3.70	sext (6.5)	–		–		–		–	
24	2.15	s	2.13	s	1.10	d (6.5)	–		–		–		–	

[a] Measured in chloroform-*d*.
[b] Measured in acetone-*d*$_6$.

The NMR spectra (Tables 1 and 2) exhibited signals attributable to an α-methyl-α,β-unsaturated–γ–lactone moiety [δ_H 2.23 (3H, d, *J*=2.0 Hz, H-7), 5.12 (1H, m, H-2); δ_C 11.1 (C-7), 81.6 (C-2), 139.3 (C-4), 147.2 (C-3), 172.9 (C-5)] and an aliphatic side chain [δ_H 1.25–1.28 (m, -CH$_2$-), 1.58 (m, H-8), 2.11 (m, H-8), 2.15 (s, terminal -CH$_3$), 2.43 (t, *J*=7.5 Hz, H-22); δ_C 24.0, 24.8, 29.3–29.9, 32.9, 43.9 (-CH$_2$-), 30.0 (terminal -CH$_3$)]. Despite the absence of the signal at δ_C 210.6 in the ^{13}C NMR spectrum of compound **1**, the heteronuclear multiple bond connectivity (HMBC) correlations from H-22 (δ_H 2.43) and H-24 (δ_H 2.15) to a carbon signal at δ_C 210.6 confirmed the presence of a ketone group at C-23 of the aliphatic side chain of **1**. HMBC experiments also showed correlations of signal at δ_H 2.23 of the α-methyl group to signals of neighboring carbons C-5 (δ_C 172.9), C-4 (δ_C

139.3), C-3 (δ_C 147.2). The signal of the oxygenated methine at δ_H 5.12 (H-2) also gave cross-peak with the signal of the latter carbon. Complete analysis of the heteronuclear single quantum coherence (HSQC), HMBC data as well as combining the HR-MS for **1** resulted in its planar structure as 2,5-dihydro-4-methyl-5-oxo-2-(16-oxoheptadecyl)-3-furancarboxylic acid. This structure was similar to that of isomuronic acid, isolated from *Neuropogon trachycarpus* [9], but containing two more methylene units in its side chain.

The absolute configuration of the sole stereogenic centre C-2 in **1** was determined to be *R* by comparison of its ultraviolet circular dichroic (CD) spectral data with that reported for the one of isomuronic acid which showed a negative maximum at around 227 nm ($\Delta\varepsilon$ = –4.6) and a positive maximum at around 258 nm ($\Delta\varepsilon$ = +1.4) [9]. The CD curve of **1** (Figure 3) revealed identical profiles with the description for the one of isomuronic acid. Compound **1** is also dextrorotatory therefore it is a homologous compound of isomuronic acid. The structure of **1** was determined as shown (Figure 1) and designated (+)-vinapraesorediosic acid A.

Compound **2** was isolated as white needles and its molecular formula was determined as $C_{24}H_{40}O_5$ through its quasi–molecular ion peak at *m/z* 409.2988 $[M+H]^+$ in the HR-ESI-MS spectrum. The comparison of spectral data, i.e. NMR, IR of **1** and **2** showed the similarity (Tables 1 and 2), except for the presence of an additional methoxy group in **2**. The ^{1}H and ^{13}C NMR spectra of **2** showed signals at δ_H 3.89 (3H, s, OCH_3) and δ_C 52.3 (OCH_3), characteristics of a methyl ester. In the HMBC spectrum of **2**, the signal of this methoxy group (δ_H 3.89) gave a cross peak to C-6 (δ_C 162.7) indicating that the methoxy group was at C-6. The 2D NMR experiments proved the proposed structure to be correct and allowed the completed characterization of compound **2** depicted in Figure 1.

The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **1**, including signals of the α-methyl-α,β-unsaturated-γ-lactone and the aliphatic side chain which were presented in Tables 1 and 2. However, some chemical shift differences were observed due to a modification of the terminal side chain. A sharp singlet (3H, H-24) at δ_H 2.15 as well as a triplet (2H, *J* = 7.5 Hz, H-22) at δ_H 2.43 corresponding to the terminal 2-oxopropyl group of the aliphatic side chain of compound **1** were replaced by signals at δ_H 1.10 (3H, d, *J* = 6.5 Hz), 3.70 (1H, sext, *J* = 6.5 Hz) and 2.05 (2H, m) for a terminal 2-hydroxypropyl group of the side chain of **3**. The comparison of ^{13}C-NMR data of **3** and **1** showed good compatibility except for the replacement of the signal of the carbonyl group at δ_C 210.6 (C-23) in **1** by the signal of a hydroxylated secondary carbon at δ_C 68.0 in **3**. This structure was further confirmed by the HR-ESI-MS of **3** which showed a typical quasi–molecular ion peak at *m/z* 419.2754 $[M+Na]^+$ (Calcd. for $C_{23}H_{40}O_5Na$, 419.2775). This value was two atomic mass units more than that of compound **1**.

The absolute configuration of C-23 for the terminal 2-hydroxypropyl group of the side chain of **3** could be determined by using the Mosher ester procedure [10]. In fact, this methodology was applied to determine the absolute configuration of the terminal secondary carbinol carbon of the side chain in murolic acid and its enantiomer, protoconstipatic acid [11]. Unfortunately, after the biological assay, compound **3** was finished, therefore the absolute configuration of C-23 could not be confirmed. On the basis of these observations, 2,5-dihydro-4-methyl-5-oxo-2-(16-hydroxyheptadecyl)-3-furancarboxylic acid or vinapraesorediosic acid B (**3**) proved to be a new natural compound.

Examination of NMR, IR, MS, and physical data of compound **4** and its methyl ester **5** showed that they were in good agreement with those reported for (+)-praesorediosic acid, isolated by David *et al.* from this lichen.[8] The HMBC correlations between the methoxy group at δ_H 3.88 (3H, s) to signal at δ_C 162.8 (C-6) in compound **5** indicated the location of a methoxy group at C-6. Detailed 2D NMR analysis and comparison with the reported data [8] led us to determine the structure of compounds **4** and **5** as praesorediosic acid and 6-methyl praesorediosate, respectively.

Compound **6** was isolated as white needles and its mass spectrum showed a quasi–molecular ion peak at *m/z* 405.2200 $[M+Na]^+$ corresponding to the molecular formula of $C_{21}H_{34}O_6$ which was identical to **4**. The ^{1}H and ^{13}C NMR spectra of **6** were also similar to those of **4**, including signals of an α-methyl-α,β-unsaturated-γ-lactone and a side chain. But there were two differences in their aliphatic

side chains. The terminal carboxyl group in the side chain of **4** was replaced by a methoxycarbonyl group and the length of the chain of **6** is one methylene unit less than that of **4**. This was proved by the presence of an additional singlet signal at δ_H 3.68 (3H, OCH_3) in the ^{1}H NMR spectrum as well as a further signal at δ_C 51.7 in the ^{13}C NMR spectrum of **6**. The presence of the terminal methoxycarbonyl group in the side chain of **6** was proved by the HMBC correlations between signals of the methoxy group at δ_H 3.68 (OCH_3) as well as the two-proton triplet at δ_H 2.32 (H-20) and the carboxyl carbon at δ_C 175.0 (C-21). The HMBC experiments also confirmed the presence of a carboxyl group at C-3 and a methyl group at C-4 of the lactone ring as normal by correlations between the methyl signal at δ_H 2.24 (H-7) and signals at δ_C 172.9 (C-5), 139.6 (C-4), 147.3 (C-3) and by four bond correlations to signals at δ_C 81.5 (C-2) and 165.9 (carboxyl carbon, C-6).

Table 2. ^{13}C NMR data of compounds **1–7** (δ in ppm).

No	1[a]	2[a]	3[b]	4[b]	5[a]	6[a]	7[a]
2	81.6	81.4	82.4	81.8	81.6	81.5	81.5
3	147.2	147.6	150.0	149.4	147.8	147.3	147.8
4	139.3	137.5	137.5	137.3	137.6	139.6	137.6
5	172.9	172.9	173.8	173.4	173.1	172.9	173.0
6	164.9	162.7	164.4	163.9	162.8	165.9	162.9
6-OCH_3	–	52.3	–	–	52.5	–	52.4
7	11.1	10.8	11.2	10.8	11.0	11.1	11.0
8	32.9	32.8	33.8	33.4	33.0	32.9	32.9
9	24.8	24.7	27.1	25.5	24.8	25.1	24.9
10–18	29.3–29.9	29.2–29.6	29.9–30.8	29.4–30.4	29.2–29.7	29.3–29.7	29.5–29.1
19						24.9	25.1
20				25.7	24.9	34.3	34.3
21	24.0	23.9	25.9	34.3	34.1	175.0	174.5
21-OCH_3	–	–	–	–	–	51.7	51.5
22	43.9	43.8	40.7	174.7	179.5	–	–
23	210.6	209.3	68.0	–	–	–	–
24	30.0	29.8	24.4	–	–	–	–

[a] Measured in chloroform-*d*.
[b] Measured in acetone-*d*$_6$.

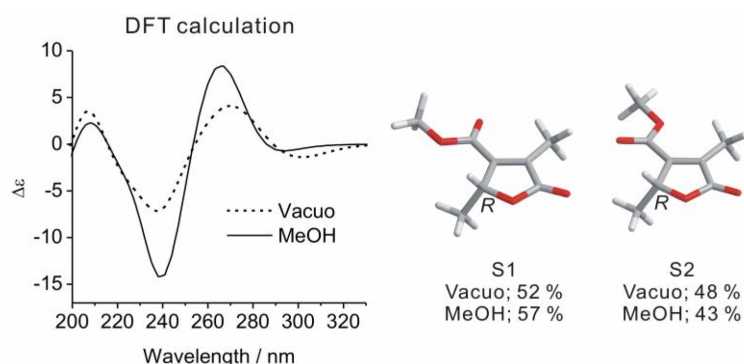

Figure 2. CD spectra (left) and two stable conformers S1 and S2 (right) for a model structure of the lactonic acids in the 4*R* configuration, calculated with DFT/B3LYP/6-311++G** (Vacuo) and CPCM(MeOH). TD-DFT method was applied for the CD calculations. The spectra were weighted-averaged for the two conformers with the simulated populations at 300 K

To confirm the proposed structure, compound **6** was esterified with methanol, ethanol and *n*-propanol to give **7**, **8** and **9**, respectively proved the proposed structure of **6** to be correct. This showed that under the acidic condition, the lactone ring in **6** could not be opened but transesterification with the solvent could occur at C-21, therefore, the obtained products possessed two ester functional

groups. These data identified compound **6** as 2,5-dihydro-4-methyl-5-oxo-2-(13-methoxycarbonyltridecyl)-3-furancarboxylic acid or vinapraesorediosic acid C.

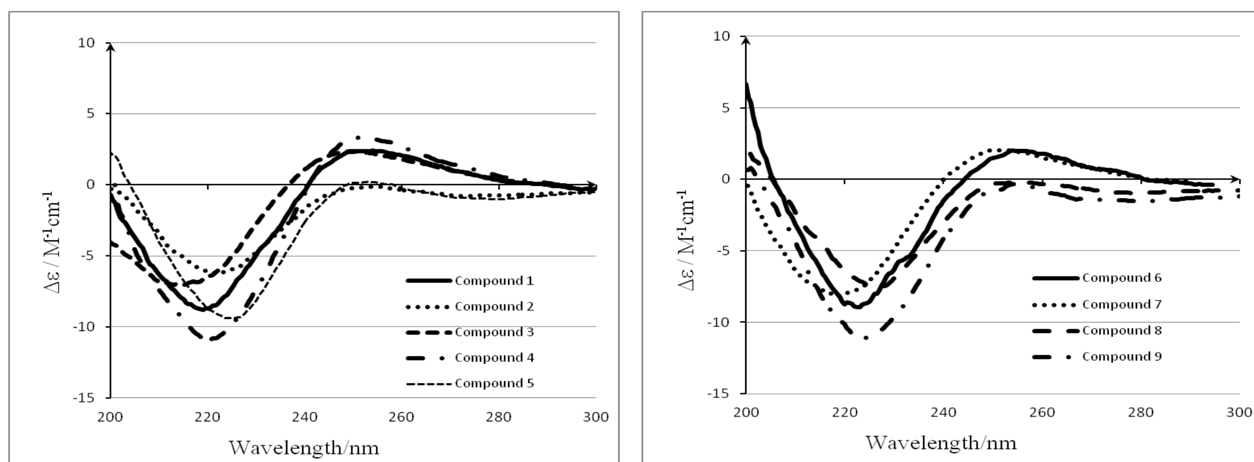

Figure 3. CD spectra of compounds **1–9**

Table 3. Cell growth inhibitory effects of the purified compounds.

Compound[a]	Inhibition of Cell Growth (I %)		
	HeLa	NCI-H460	MCF-7
Vinapraesorediosic acid A (**1**)	40.3±0.5[b]	39.3±4.0	52.5±2.3
6-Methyl vinapraesorediosate A (**2**)	13.5±6.7	22.0±2.4	25.3±10.9
Vinapraesorediosic acid B (**3**)	17.0±1.1	-4.1±1.3	26.8±4.9
Praesorediosic acid (**4**)	0.1±0.3	-1.9±0.9	-3.7±1.4
6-Methyl praesorediosate (**5**)	2.3±2.0	-2.5±4.9	0.2±5.7
Vinapraesorediosic acid C (**6**)	-6.4±1.8	-12.9±5.1	-3.3±3.7
Camptothecin (positive control)[c]	58.2±3.3	77.6±0.6	41.2±2.4

[a] The compounds were tested at the concentration of 100 µg/mL.
[b] The presented data are means of three experiments ± S.D.
[c] Camptothecin was tested at the concentration of 0.01 µg/mL for MCF-7 and NCI-H460 and of 1 µg/mL for HeLa.

The testing of cytotoxic activities of isolated compounds against the MCF-7 (breast cancer cell line), HeLa (cervical cancer cell line) and NCI-H460 (lung cancer cell line) was done using the sulforhodamine B method (SBR assay) [12]. The results were presented in Table 3. Among six compounds, only **1** possessed medium activity against MCF-7 cell line with the inhibition of 52.5% at the dose of 100 µg/mL. Meanwhile, the rest showed no cytotoxic activity against three surveyed cancer cell lines. Their poor activities could originate from the presence of the double bond in the γ–butyrolactone [6-7].

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] S. Huneck and I. Yoshimura (1996). Identification of Lichen Substances. Springer-Verlag: Berlin, Heidelberg, New York. 140–152.

[2] S. Huneck and G. Hofle (1980). Structure of acaranoic and acarenoic acids, *Phytochemistry* **19**, 2713–2715.

[3] S. Huneck, T. Tϕnsberg, F. and Bohlmann (1986). (−)-Allo-pertusaric acid and (−)-dihydropertusaric acid from the lichen *Pertusaria albescens*, *Phytochemistry* **25**, 453–459.

[4] A. W. Archer and W. C. Taylor (1987). Homoheveadride, a cyclononadiene bis-anhydride from *Cladonia polycarpoides, Phytochemistry* **26**, 2117–2119.

[5] B. B. Cleber and L. P. P. Vera (2009). A formal synthesis to (+)-nephrosteranic acid from chiral nitroalkyl derivatives, *Tetrahedron Lett.* **50**, 6389–6392.

[6] C. Andrea, F. Cristina, N. Patrizia, P. Giuliana and V. Ennio (2004). Chemoenzymatic synthesis of enantioenriched 5-oxo-tetrahydro-3-furancarboxylic acid derivatives, *Tetrahedron-Asymmetry.* **15**, 617–625.

[7] A. J. Peter and H. Prudencio (1996). Enantioselective syntheses of (+)- and (-)-phaseolinic acid, *Tetrahedron Lett.* **37**, 8297–8300.

[8] F. David, J. A. Elix and M. W. B. Samsudin (1990). Two new aliphatic acids from the lichen *Parmotrema praesorediosum, Aust. J. Chem*. **43**, 1297–1300.

[9] B. Bodo and D. Molho (1980). Structure des acides isomuronique et neuropogolique, nouveaux acides aliphatiques du lichen *Neuropogon trachycarpus*, *Phytochemistry* **19**, 1117–1120.

[10] E. Antonio, S. Lorenzo, F. Olga, B. Giovanni and M. Andrea (1999). Sapinofuranones A and B: Two new 2(3*H*)-dihydrofuranones produced by *Sphaeropsis sapinea*, a common pathogen of conifers, *J. Nat. Prod*. **62**, 253–256.

[11] T. Rezanka and I. A. Guschina (2000). Glycosidic compounds of murolic, protoconstipatic and allo-murolic acids from lichens of Central Asia, *Phytochemistry* **54**, 635–645.

[12] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd (1990). New colorimetric cytotoxicity assay for anticancer-drug screening, *J. Natl. Cancer Inst*. **82**, 1107–1112.

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