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Total synthesis and biological evaluation of (+)- and (−)-Butyl ester of rosmarinic acid

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An efficient method for the synthesis of the natural product (+)-(R)-butyl ester of rosmarinic acid **(+)-(R)-1** and its enantiomer **(−)-(S)-1** has been developed by chemical resolution of its phenyl lactic acid precursors **4** with (−)-menthol. Their antioxidative and anti-tumor activities were evaluated.

Keywords: (+)- and (−)-Butyl ester of rosmarinic acid; Chemical resolution

1. Introduction

(+)-(R)-Butyl ester of rosmarinic acid **(+)-(R)-1** (figure 1) isolated from *Isodon oresbius* in 1999 [1] was a derivative of rosmarinic acid which possesses various biological activities such as antioxidant [2], anti-HIV [3] and anti-inflammatory effects [4].

Two synthetic routes of the skeleton of rosmarinic acid have been reported [5,6]. In order to establish the chiral center, the expensive chiral material tyrosine was used in one route [5]; the method of chemoenzymatic resolution was used in another route [6]. In an earlier report, we have described the synthetic route of racemic compound **1** in moderate yield [7]. The following contribution is dedicated to the efficient synthesis of optically active form **(+)-(R)-1** and **(−)-(S)-1** (figure 1) through the chemical resolution of its phenyl lactic acid precursors **4** with (−)-menthol.

2. Results and discussion

(+)-(R)-1 and **(−)-(S)-1** were synthesized via piperonal **2** as a starting material in seven steps (scheme 1).

Piperonal **2** was reacted with excess of aceturic acid in the presence of anhydrous NaOAc in Ac_2O to give azalactone **3**. We adopted 'one-pot' procedure in which **3** was first refluxed with 3 mol/L hydrochloric acid, subsequent addition of excess zinc amalgam to

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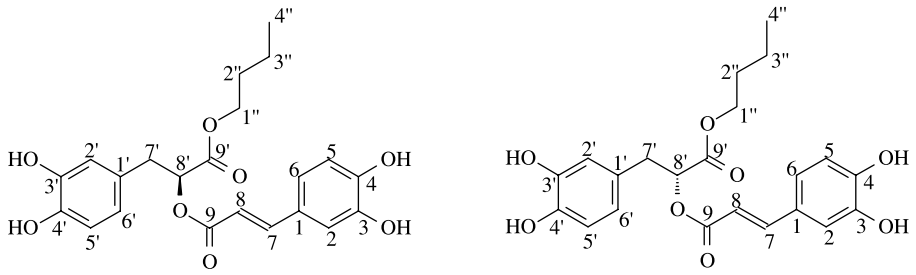
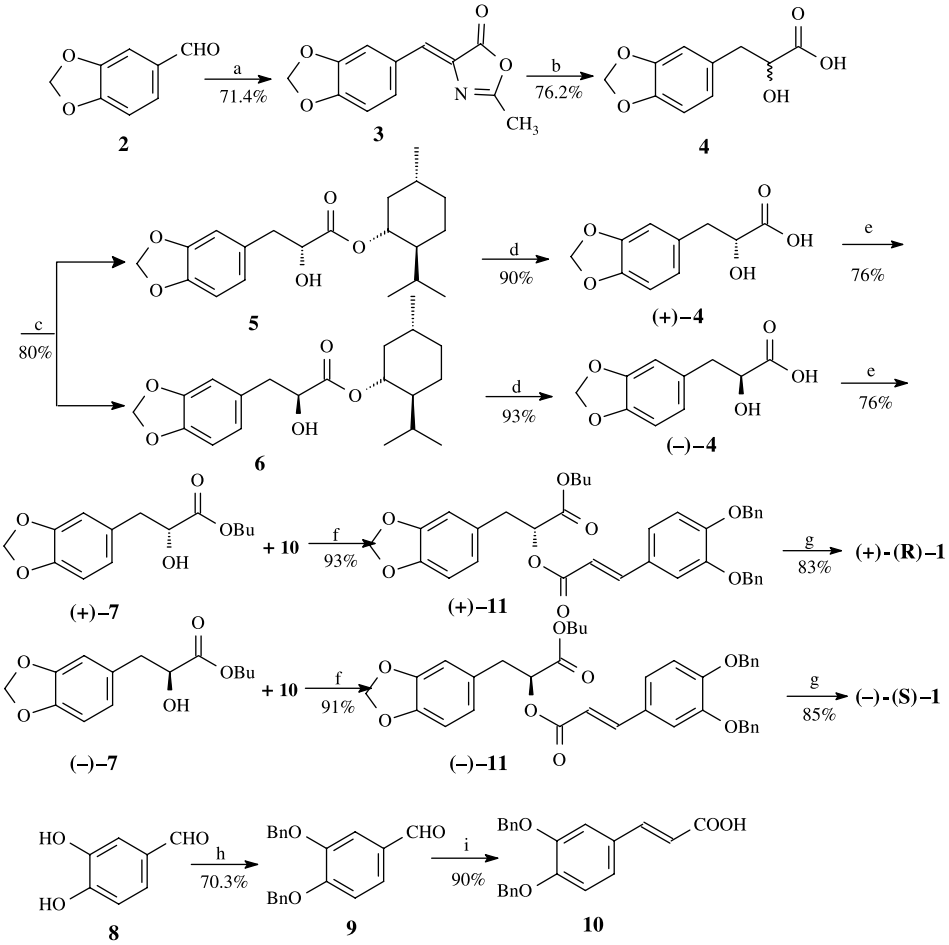


Figure 1. Absolute configuration of compound **1**.



Scheme 1. Synthesis of (+)- and (−)-**1**. Regents and conditions: (a) aceturic acid, Ac_2O, NaOAc, 120°C, 3.5 h; (b) HCl, 100°C, 4 h, then Zn/Hg, HCl, 3 h; (c) H_2SO_4, CH_2Cl_2, (−)-menthol, 24 h, column chromatography; (d) NaOH, THF/CH_3OH/H_2O, reflux, 2 h: (e) H_2SO_4, CH_2Cl_2, n-BuOH, 24 h; (f) DCC, DMAP, CH_2Cl_2, −20°C, 10 h; (g) BBr_3, −78°C, 1.5 h; (h) K_2CO_3, ethanol, $PhCH_2Cl$, reflux, 5 h; (i) malonic acid, pyridine, piperidine, 110°C, 3 h.

Table 1. Anti-tumor activities of **(+)-(R)-1, (−)-(S)-1** and **(±)-1** against human colon cancer (HT-29), ovary cancer (A2780) and melanin cancer (A2375) cell lines *in vitro*.

	IC_{50} (Mol/L)		
Compound	*HT-29*	*A2375*	*A2780*
(±)-1	2.53×10^{-4}	1.38×10^{-3}	2.38×10^{-3}
(+)-(R)-1	3.02×10^{-4}	5.61×10^{-4}	8.35×10^{-3}
(−)-(S)-1	2.21×10^{-3}	>1	>1

give **4**. **(+)-** and **(−)-4** were obtained by resolution with (−)-menthol through the intermediates **5** and **6**. Absolute configuration of **(+)-** and **(−)-4** was determined to R and S by comparison of the optical rotations with the known values of R- and S-3- (3,4-dihydroxyphenyl) lactic acid, respectively [5,8]. The key intermediates **(+)-** and **(−)-7** were obtained by esterification of **(+)-** and **(−)-4** with n-BuOH, respectively. Esterification of **(+)-** and **(−)-7** with **10** which was obtained from **8** via intermediate **9** produced **(+)-** and **(−)-11** in 93% and 91% yield, respectively. The title compounds **(+)-(R)-1** and **(−)-(S)-1** were obtained by treating **(+)-** and **(−)-11** with BBr_3 in *ca* 80% yield.

Compounds **(+)-(R)-1**, **(−)-(S)-1** and **(±)-1** were evaluated for their anti-tumor and antioxidative activities (tables 1 and 2). **(±)-1** and **(+)-(R)-1** showed the similar activities against human colon cancer (HT-29), ovary cancer (A2780), melanin cancer (A2375) cell lines. In particular, **(+)-(R)-1** showed 10-fold, 10^4-fold and 10^3-fold better activities than **(−)-(S)-1** against the above-mentioned three cell lines, respectively. The results indicated that the configuration of chiral carbon might be a playing crucial role for the anti-tumor activities. The antioxidative activities of compounds **(+)-(R)-1**, **(−)-(S)-1** and **(±)-1** were compared with V_E as reference. All the three compounds exhibited good inhibition on Fe^{2+} induced lipid peroxidation (malondialdehyde formation) in rat liver microsomes *in vitro*. The inhibitory effects are equal to V_E.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a XT_4-100_X micro-melting apparatus and are uncorrected. IR spectra were run on a NICOLET IMPACT-400 spectrometer. Optical

Table 2. Effects of **(+)-(R)-1, (-)-(S)-1**, **(±)-1** and V_E on cysteine-Fe^{2+} induced malondialdehyde formation in rat liver microsomes *in vitro*.

Compound	*mol/L*	*Inhibition rate (%)*
(±)-1	10^{-4}	91.7
	10^{-5}	81.6
	10^{-6}	56.6
(+)-(R)-1	10^{-4}	91.7
	10^{-5}	81.1
	10^{-6}	49.0
(−)-(S)-1	10^{-4}	91.4
	10^{-5}	78.0
	10^{-6}	47.5
V_E	10^{-4}	97.5
	10^{-5}	62.6
	10^{-6}	57.3

rotations were measured on PE-241 digital polarimeter. NMR spectra were recorded on Varian Mercury-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts of ^{1}H and ^{13}C spectra are referenced to the NMR solvents. Mass spectra were obtained on a ZAB-2F spectrometer. TLC was carried out on silica gel (GF_{254}). Column chromatography was run on silica gel (200–300 mesh) from Qingdao Ocean Chemical Factory. Dichloromethane was distilled over P_2O_5.

3.2 General procedures for the synthetic compounds

3.2.1 Compounds 5 and 6. To a solution of **4** (1.0 g, 4.8 mmol) and (−)-menthol (0.9 g, 5.8 mmol) in 30 mL CH_2Cl_2, 5 drops concentrated H_2SO_4 were added. The mixture was stirred at room temperature for 24 h. Water (10 mL) was added and the organic phase was washed with water (2 × 10 mL), dried over Mg_2SO_4 and evaporated to give the crude product (1.7 g) which was purified by column chromatography (PE: EtOAc = 20:1). The first fraction was (−)-menthol and discarded, the second fraction was compound **6** (0.7 g) as colorless oil, the third fraction was the mixture of **5** and **6** (0.37 g), the fourth fraction was compound **5** (0.6 g) as colorless needles. Compound **5**: mp 64–65°C, $[\alpha]_D^{25}$ −27.7 (c 0.66, $CHCl_3$). ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm): 6.75–6.67 (m, 3H), 5.92 (s, 2H), 4.73 (m, 1H, for menthol), 4.33 (dd, 1H, J = 6.9 Hz, 4.2 Hz), 3.06 (dd, 1H, J = 13.8 Hz, 4.2 Hz), 2.83 (dd, 1H, J = 13.8 Hz, 6.9 Hz), 2.60 (brs, 1H), 2.02 (m, 9H, for menthol). EI-MS *m/z* (%): 348 (M^+, 15), 330 (10), 192 (45), 135 (100). Compound **6**: $[\alpha]_D{}^{25}$ −59.1 (c 0.57, $CHCl_3$). ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm): 6.75–6.66 (m, 3H), 5.92 (s, 2H), 4.75 (m, 1H, for menthol), 4.37 (dd, 1H, J = 6.0 Hz, 4.2 Hz), 3.04 (dd, 1H, J = 13.8 Hz, 4.2 Hz), 2.86 (dd, 1H, J = 13.8 Hz, 6.0 Hz), 2.58 (brs, 1H), 1.94–0.72 (m, 9H, for menthol). EI-MS *m/z* (%): 348 (M^+, 15), 330 (10), 192 (45), 135 (100).

3.2.2 Compounds (+)-4 and (−)-4. The mixture of **5** (0.5 g, 1.44 mmol) or **6** (0.5 g, 1.44 mmol) in 20 mL THF/CH_3OH/H_2O (1:1:1) with NaOH (69 mg, 1.73 mmol) was refluxed for 2 h. The mixture was cooled to room temperature, acidified with ice-cold 2 mol/L HCl (5 mL) and extracted with EtOAc (3 × 20 mL), the combined organic phase was washed with water (2 × 15 mL), dried over Mg_2SO_4 and evaporated to give the crude product **(+)-4** or **(−)-4**, respectively. The crude product was recrystallized in petroleum ether and EtOAC to give **(+)-4** (0.27 g) or **(−)-4** (0.28 g) as colorless needle. Compound **(+)-4**: mp 109–110°C, $[\alpha]_D{}^{25}$ + 13.3 (c 0.66, CH_3OH). Compound **(−)-4**: mp 112–113°C, $[\alpha]_D{}^{25}$ −15.6 (c 0.41, CH_3OH). The spectral data of **(+)-4** and **(−)-4** were the same as racemic compound **4**. Absolute configuration of **(+)-4** was determined to R by comparison the optical rotation with the known value of R-3-(3,4-dihydroxyphenyl)lactic acid ($[\alpha]_D{}^{25}$ + 10.8 in CH_3OH) and that of **(−)-4** was determined to S by comparison with S-3-(3,4-dihydroxylphenyl)-lactic acid ($[\alpha]_D{}^{25}$ −10.8 in CH_3OH) [5,8].

3.2.3 Compounds (+)-7 and (−)-7. To a solution of **(+)-4** (0.21 g, 1 mmol) or **(−)-4** (0.25 g, 1.2 mmol) and n-butanol (0.15 g, 2 mmol) in 10 mL CH_2Cl_2, 3 drops concentrated H_2SO_4 was added. The mixture was stirred at room temperature for 24 h; water (5 mL) was added. The organic phase was washed with water (2 × 5 mL), dried over Mg_2SO_4 and evaporated to give the crude product **(+)-7** or **(−)-7** which was purified by column chromatography (PE: EtOAc = 7:1). **(+)-7** (0.2 g) and **(−)-7** (0.24 g) were obtained as slightly yellow oil, respectively. **(+)-7**: $[\alpha]_D{}^{25}$ + 27.3 (c 0.74, $CHCl_3$). (−)-**7**: $[\alpha]_D{}^{25}$

− 29.5 (c 1.38, $CHCl_3$). ^{1}HNMR (300 MHz, $CDCl_3$) δ (ppm): 6.73 (d,1H, $J = 7.8$ Hz), 6.71 (s,1H), 6.65 (d, 1H, $J = 7.8$ Hz), 5.92 (s, 2H), 4.38 (dd, 1H, $J = 6.6$ Hz, 4.2 Hz), 4.15 (t, 2H, $J = 6.6$ Hz), 3.03 (dd, 1H, $J = 14.1$ Hz, 4.2 Hz), 2.88 (dd, 1H, $J = 14.1$ Hz, 6.6 Hz), 1.64 (m, 2H), 1.36 (m, 2H), 0.94 (t, 3H, $J = 6.6$ Hz). EI-MS *m/z* (%): 266 (M^+, 10), 248 (5), 135 (100).

3.2.4 Compounds (+)-11 and (−)-11. To a solution of **(−)-7** (0.2 g, 0.75 mmol) in anhydrous CH_2Cl_2 (10 mL) was added **10** (0.54 g, 1.5 mmol) and DMAP (12 mg, 0.1 mmol). DCC (0.31 g, 1.5 mmol) was added at − 20°C and the mixture was allowed to room temperature within 10 h. N, N-Dicyclohexylurea was filtered and the filtrate was evaporated to give the crude product which was purified by column chromatography. **(−)-11** (0.41 g) were obtained as colorless oil. $[\alpha]_D^{25}$ − 28.1 (c 0.70, $CHCl_3$). According to the same procedure, (+)-**11** (0.31 g) was obtained as the colorless oil from **(+)-7**. $[\alpha]_D^{25}$ + 28.8 (c 0.98, $CHCl_3$). ^{1}HNMR (300MHz, $CDCl_3$): δ (ppm): 7.61 (d, 1H, $J = 15.9$ Hz, = CH −), 7.49–7.31 (m, 10H, ArH), 7.14 (d, 1H, $J = 2.0$ Hz, ArH), 7.08 (dd, 1H, $J = 8.4$ Hz, 2.0 Hz, ArH), 6.92 (d, 1H, $J = 8.4$ Hz, ArH), 6.78 (d, 1H, $J = 1.4$ Hz, ArH), 6.74 (d, 1H, $J = 8.0$ Hz, ArH), 6.70 (dd, 1H, $J = 8.0$ Hz, 1.4 Hz, ArH), 6.30 (d, 1H, $J = 15.9$ Hz, = CH −), 5.93 (s, 2H, OCH_2O), 5.30 (t, 1H, $J = 6.6$ Hz, CHO −), 5.20 (s, 2H, $-OCH_2Ph$), 5.19 (s, 2H, $-OCH_2Ph$), 4.16 (t, 2H, $J = 6.6$ Hz, $-OCH_2$ −), 3.12 (t, 2H, $J = 6.6$ Hz, CH_2Ar), 1.65–1.58 (m, 2H, CH_2), 1.41–1.28 (m, 2H, CH_2), 0.92 (t, 3H, $J = 7.2$ Hz, CH_3); IR (KBr, cm^{-1}): 1743, 1716, 1634, 1596; EI-MS: *m/z* 608 (M^+, 0.2), 91 (100).

3.2.5 Compounds (+)-(R)-1 and (−)-(S)-1. To **(−)-11** (0.35 g, 0.58 mmol) in anhydrous CH_2Cl_2 (15 mL) was added slowly BBr_3 (0.16 ml, 1.74 mmol) at − 78°C. The mixture was stirred for 1.5 h at − 78°C and at once poured into H_2O (25 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phase was dried over Mg_2SO_4 and concentrated. The crude product was purified by column chromatography (PE: EtOAC = 1:20) to give **(−)-(S)-1** (0.22 g) as slight yellow solid. $[\alpha]_D^{25}$ − 28.7 (c 0.52, CH_3OH). ^{1}HNMR (300 MHz, DMSO-d6): *(* (ppm) 7.48 (d, 1H, $J = 15.9$ Hz, H-7), 7.06 (d, 1H, $J = 1.8$ Hz, H-2), 7.04 (dd, 1H, $J = 7.8$ Hz, 1.8Hz, H-6), 6.77 (d, 1H, $J = 7.8$ Hz, H-5), 6.65 (d, 1H, $J = 1.8$ Hz, H-2′), 6.63 (d, 1H, $J = 7.8$ Hz, H-5′), 6.49 (dd, 1H, $J = 7.8$ Hz, 1.8 Hz, H-6′), 6.26 (d, 1H, $J = 15.9$ Hz, H-8), 5.08 (t, 1H, $J = 6.6$ Hz, H-8′), 4.03 (t, 2H, $J = 6.0$ Hz, H-1″), 2.95 (d, 2H, $J = 6.6$ Hz, H-7′), 1.52–1.38 (m, 2H, H-2″), 1.36–1.28 (m, 2H, H-3″), 0.84 (t, 3H, $J = 7.2$ Hz, H-4″); ^{13}CNMR (75MHz, DMSO-d6): *(* (ppm) 169.5 (C-9′), 165.9 (C-9), 148.6 (C-4), 146.3 (C-3), 145.5 (C-7), 144.9 (C-3′), 144.1 (C-4′), 125.6 (C-1′), 125.3 (C-1), 121.7 (C-6), 120.1 (C-6′), 116.7 (C-2′), 115.7 (C-5), 115.4 (C-5′), 114.9 (C-2), 112.9 (C-8), 72.9 (C-8′), 64.4 (C-1″), 36.2 (C-7′), 30.0 (C-2″), 18.4 (C-3″), 13.5 (C-4″); IR (KBr, $cm^{-1)}$: 3379, 1716, 1604; FAB-MS: *m/z* 417 (M^+ + H, 0.1), 163 (100). HRFAB-MS: *m/z* 417.1573 $[M + H]^+$ (calcd for $C_{22}H_{25}O_8$, 417.1549). **(+)-(R)-1** was prepared from **(+)-11** according to the same procedure: $[\alpha]_D^{25}$ + 27.6 (c 0.34, CH_3OH). FAB-MS: *m/z* 417 (M^+ + H, 0.1), 163 (100). HRFAB-MS: *m/z* 417.1534 $[M + H]^+$ (calcd for $C_{22}H_{25}O_8$, 417.1549).

3.2.6 Compounds 3, 4 and 10. Azalactone **3** was prepared from piperonal **2** according to Erlenmeyer-PlÖchl method [9,10]. **4** was obtained from **3** according to the literature [11]. **10** was easily prepared from **9** (obtained from the corresponding phenolic benzaldehyde **8** by reaction with benzyl chloride in ethanol) by Knoevenagel reaction [12]. All the spectral data of compounds **3**, **4** and **10** are compatible with the reported data [10–12].

3.3 Biological evaluation

3.3.1 Anti-tumor activities. Anti-tumor activities of **(+)-(R)-1, (−)-(S)-1** and **(±)-1** against human colon cancer (HT-29), ovary cancer (A2780), melanin cancer (A2375) cell lines were evaluated using the MTT assay. The results are given as IC_{50} values and are shown in table 1.

3.3.2 Antioxidative activities. The antioxidative effects of **(+)-(R)-1**, **(−)-(S)-1** and **(±)-1** have been investigated with V_E as reference. All the three compounds were found to inhabit Fe^{2+} induced lipid peroxidation (malondialdehyde formation) in rat liver microsomes *in vitro*. The results are shown in table 2.

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