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 (1)(+)-(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 (1)(+)-(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 Ac₂O 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
Figure 1. Absolute configuration of compound 1.

Scheme 1. Synthesis of (+)- and (−)-1. Regents and conditions: (a) aceturic acid, Ac₂O, NaOAc, 120°C, 3.5 h; (b) HCl, 100°C, 4 h, then Zn/Hg, HCl, 3 h; (c) H₂SO₄, CH₂Cl₂, (−)-menthol, 24 h, column chromatography; (d) NaOH, THF/CH₃OH/H₂O, reflux, 2 h; (e) H₂SO₄, CH₃Cl₂, n-BuOH, 24 h; (f) DCC, DMAP, CH₂Cl₂, −20°C, 10 h; (g) BBr₃, −78°C, 1.5 h; (h) K₂CO₃, ethanol, PhCH₂Cl, reflux, 5 h; (i) malonic acid, pyridine, piperidine, 110°C, 3 h.
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 BBr3 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, 104-fold and 103-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 Fe²⁺ 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 XT₄-100X micro-melting apparatus and are uncorrected. IR spectra were run on a NICOLET IMPACT-400 spectrometer. Optical
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\(_2\)O\(_5\).

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\(_2\)Cl\(_2\), 5 drops concentrated H\(_2\)SO\(_4\) 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 \(\times\) 10 mL), dried over Mg\(_2\)SO\(_4\) 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: m.p 64 – 65°C, \([\alpha]_{D}^{25}\) 27.7 (c 0.66, CHCl\(_3\)). 1HNMR (300 MHz, CDCl\(_3\)) \(\delta\) (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).

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\(_3\)OH/H\(_2\)O (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 \(\times\) 20 mL), the combined organic phase was washed with water (2 \(\times\) 15 mL), dried over Mg\(_2\)SO\(_4\) 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\(_3\)OH). Compound (-)-4: mp 112–113°C, \([\alpha]_{D}^{25}\) –15.6 (c 0.41, CH\(_3\)OH). 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\(_3\)OH) and that of (-)-4 was determined to S by comparison with S-3-(3,4-dihydroxyphenyl)-lactic acid (\([\alpha]_{D}^{25}\) 10.8 in CH\(_3\)OH) [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\(_2\)Cl\(_2\), 3 drops concentrated H\(_2\)SO\(_4\) 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 \(\times\) 5 mL), dried over Mg\(_2\)SO\(_4\) 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: \([\alpha]_{D}^{25}\)
3.2.4 Compounds (+)-11 and (−)-11. To a solution of (−)-7 (0.2 g, 0.75 mmol) in anhydrous CH2Cl2 (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. [α]D25 28.1 (c 0.70, CHCl3). According to the same procedure, (+)-11 (0.31 g) was obtained as the colorless oil from (+)-7. [α]D25 28.8 (c 0.98, CHCl3).

3.2.5 Compounds (+)-(R)-1 and (−)-(S)-1. To (−)-11 (0.35 g, 0.58 mmol) in anhydrous CH2Cl2 (15 mL) was added slowly BBr3 (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 H2O (25 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phase was dried over MgSO4 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. [α]D25 28.7 (c 0.52, CH2OH).

3.2.6 Compounds 3, 4 and 10. Azalactone 3 was prepared from piperonal 2 according to Erlenmeyer-Pflüchter 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 VE 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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References
