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Targeted acylation for all the hydroxyls of (+)-catechin and evaluation of their individual contribution to radical scavenging activity

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ABSTRACT

The reactivity profile of all the hydroxyl groups in (+)-catechin towards acylation and their respective contribution to radical scavenging activity were systematically explored in this work. Selective acylation of the hydroxyls on different rings was carried out employing either a basic or acidic activation strategy. Monoacylation of B ring was achieved effectively with the aid of dimethyltin dichloride. Monoacylation of A ring was accomplished by sequential protection and deprotection of B and C rings. Based on specific acylation of all the individual hydroxyls of (+)-catechins, the structure radical scavenging activity relationship of each hydroxyl of (+)-catechin was established. It was demonstrated that the vicinal phenolic hydroxyls on B ring played the most important role in the ABTS radical scavenging activity and those on A and C rings made a much smaller contribution. This study has laid solid groundwork for further modification of the catechins and improvement of their properties.

1. Introduction

As one type of flavan-3-ol natural products, catechins are of particular importance owing to their health-beneficial effects in many foods especially green tea (Raab et al., 2010). A significant reduction in cardiovascular diseases and cancers has been associated with green tea consumption (Cabrera, Artacho, & Giménez, 2006; Cao & Cao, 1999). Because of the versatile biological roles of catechins, tremendous efforts have been made to develop modification methods for catechin molecules (Fukuhara et al., 2009; Kurisawa, Chung, Uyama, & Kobayashi, 2004; Manda et al., 2008; Ohmori, Shono, Hatakoshi, Yano, & Suzuki, 2011; Stadlbauer, Ohmori, Hattori, & Suzuki, 2012; Watanabe, Ohmori, & Suzuki, 2013; Yoshida et al., 2011). Among the various modification methods, acylation of catechins has been extensively applied to improve their stability, bioavailability and bioactivity. Nicolosi et al. (Patti, Piattelli, & Nicolosi, 2000) developed a straightforward enzymatic preparation of catechin esters bearing a long-chain acyl group at the C-3 position which were more lipophilic than the parent catechin and applicable as antioxidants in hydrophobic matrices. Noteworthily, Dou et al. (Landis-Piwowar et al., 2007) significantly promoted the stability, bioavailability, proteasome inhibitory and anticancer activities of (-)-epigallocatechin-3-gallate in human breast cancer cells and tumors, through introduction of peracetate protection of its hydroxyl groups. Shahidi et al. (Zhong & Shahidi, 2011) demonstrated that esterification of epigallocatechin-3-gallate with long-chain saturated or polyunsaturated fatty acids resulted in catechin derivatives with higher lipophilicity and greater antioxidant activity.

However, although many studies on the modification of catechins have been carried out, specific acylation of each hydroxyl in the molecules has not been systematically investigated, which is essential for further establishment of a molecular structure activity relationship and optimization of molecular properties. In our group, research is focusing on the development of a synthetic methodology for polyphenols and optimization of their properties. In a previous study, the novel chemical synthesis of phenolic ginkgolic acid (13:0) was developed employing a state-of-the-art catalytic cross-coupling reaction to build the long side chain from naturally abundant 2,6-dihydroxybenzoic acid (Fu, Hong, Li, & Liu, 2013). We also accomplished the simultaneous preparation of naturally abundant and rare catechins, (-)-epigallocatechin gallate, (-)-epicatechin gallate, (-)-epicatechin, and (-)-epigallocatechin of high purity by tannase-mediated biotransformation combining high speed counter current chromatography (Xia, Hong, & Liu, 2014).

In continuation of our catechin study, we are interested in the specific role of each hydroxyl group of catechins in regards to antioxidant activity and other bioactivities, which requires a

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systematic exploration of the reactivity profile of the hydroxyl groups in the molecules to achieve specific manipulation of each hydroxyl. To realize this goal, we initiated specific acylation and structure activity relationship studies on the basic structure of catechins, (+)-catechin. In this study, a reactivity profile of the hydroxyl groups in (+)-catechin towards acylation was systematically explored, and the structure antioxidant activity relationship of all the hydroxyls were thoroughly investigated employing an ABTS assay.

2. Materials and methods

2.1. Chemicals

(+)-Catechin hydrate was purchased from Sigma–Aldrich. Acetic anhydride and acetonitrile were purchased from Shanghai Lingfeng Chemical Reagent Co. Ltd. (China) and Tianjin Shield Fine Chemicals Company (China), respectively. Propionic anhydride, *n*-butyric anhydride, isobutyric anhydride, benzyl chloroformate (CbzCl), triethylamine, *N,N*-dimethylaminopyridine (DMAP), Pd/C, triethylsilane, dimethyltin dichloride, pyridine, *p*-toluenesulfonic acid (TSA), dihydropyran (DHP), and dodecanoyl chloride were purchased from Aladdin-Reagent (China) and used as obtained. Solvents (tetrahydrofuran (THF), MeCN, $CHCl_3$, CH_2Cl_2, MeOH and tert-butyl methyl ether (*t*-BME) were AR grade and purchased from Sinopharm. Hexanes and ethyl acetate for extraction and chromatography were used as purchased.

2.2. Synthesis of 3-dodecanoyl (+)-catechin (compound 1)

To a solution of (+)-catechin (435.5 mg, 1.50 mmol) in THF (25 ml) was added pyridine (2.42 ml, 30.0 mmol) and DMAP (9.6 mg, 0.075 mmol), and then dodecanoyl chloride (5.20 ml, 22.5 mmol) was dripped into the stirred mixture. The reaction was allowed to stand at ambient temperature for 24 h (Raab et al., 2010). Then the reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography (SiO_2; elution:hexanes/ethyl acetate, 10:1) was applied to obtain the fully acylated product as a light yellow powder.

The above prepared product (1.623 g, 1.35 mmol) was dissolved in a mixture of methanol (12 ml) and THF (12 ml), and treated with hydrazine (524.0 μl, 10.80 mmol). The reaction mixture was stirred at 25 °C for 8 h and then extracted with ethyl acetate and evaporated (Khan, 1995). Purification of the crude product by chromatography (SiO_2; elution:chloroform/methanol, 15:1) produced compound **2** as a light yellow powder.

2.3. Synthesis of 3′-dodecanoyl (+)-catechin and 4′-dodecanoyl (+)-catechin (compound 2 and 3)

(+)-Catechin (145.7 mg, 0.5 mmol) was dissolved in THF (7.5 ml), followed by sequential addition of $NaHCO_3$ (84.6 mg, 1.0 mmol) and dimethyltin dichloride (Me_2SnCl_2) (132.3 mg, 0.6 mmol). After the solution was stirred at ambient temperature for 20 min, dodecanoyl chloride (173.6 μl, 0.75 mmol) was slowly dripped into the mixture (Iwasaki, Maki, Onomura, Nakashima, & Matsumura, 2000; Maki, Iwasaki, & Matsumura, 1998). Then the reaction was terminated after 7 h. The crude product afforded by aqueous work-up and EtOAc extraction was purified by chromatography (SiO_2; elution:chloroform/methanol, 15:1) and provided the mixture of compound **2** and **3** as light yellow power.

The separation of **2** and **3** was performed employing an Agilent Technologies PreStar HPLC system (Agilent, Santa Clara, CA). A C18 column (20 ID × 250 mm, COSMOSIL 5C18-MS-II, Japan) operated at 30 °C was applied, and the sample was eluted with an isocratic solvent system containing 0.1% aqueous formic acid/acetonitrile (40:60) at a flow rate of 10 ml/min.

2.4. Synthesis of 5-dodecanoyl (+)-catechin and 7-dodecanoyl (+)-catechin (compound 4 and 5)

To a solution of (+)-catechin (639.0 mg, 2.20 mmol) in THF (33 ml) was added *p*-toluenesulfonic acid (84.0 mg, 0.44 mmol) and dihydropyran (602.0 μl, 6.60 mmol) (Bernady, Floyd, Poletto, & Weiss, 1979). After stirring for 40 min at ambient temperature, the reaction mixture was extracted with EtOAc, washed with water and dried over $MgSO_4$. The afforded syrup after evaporation was subjected to flash chromatography purification (SiO_2; elution:chloroform/methanol, 20:1) to give the product **a1** as a light yellow solid (418.8 mg).

The prepared **a1** (418.8 mg, 1.12 mmol) was dissolved in acetonitrile (25 ml), followed by addition of CbzCl (305.4 μl, 2.24 mmol) and Et_3N (390.2 μl, 2.80 mmol) (Vandyk, Steynberg, Steynberg, & Ferreira, 1990). The reaction mixture was stirred at 0 °C for 2 h before extraction with EtOAc, and washed by 1 N aqueous HCl. The crude product was purified by flash chromatography (SiO_2; elution:chloroform/methanol, 20:1) to afford **a2** (431.3 mg).

To **a2** (431.3 mg, 0.67 mmol) in *t*-BME (15 ml) was sequentially added pyridine (189.2 μl, 2.35 mmol), DMAP (4.4 mg, 0.03 mmol) and dodecanoyl chloride (170.8 μl, 0.74 mmol) (Raab et al., 2010). The reaction mixture was stirred at ambient temperature 5 h and then extracted with EtOAc, washed by 1 N HCl, and dried over $MgSO_4$. After evaporation of the solvent, the afforded product was subjected to flash chromatography purification (hexanes: EtOAc, 5:1 to 3:1) to give the product as a mixture of **4a** and **5a** (138.4 mg).

The resultant mixture of **4a** and **5a** (138.4 mg, 0.17 mmol) was treated with *p*-toluenesulfonic acid (31.9 mg, 0.17 mmol) in isopropanol (3.5 ml) at 25 °C for 3 h (Almqvist & Frejd, 1995). The obtained product (**4b** and **5b**) was dissolved in MeOH (3.0 ml), and treated with triethylsilane (313.8 μl, 1.96 mmol), Et_3N (36.5 μl, 0.26 mmol) and Pd/C (5%) (13.9 mg, 0.0065 mmol) under an N_2 atmosphere (Coleman & Shah, 1999). After stirring at ambient temperature for 12 h, the reaction mixture was filtered and then extracted with EtOAc, then washed by 1 N HCl. The crude product was subjected to flash chromatography purification (silica gel; chloroform:methanol, 15:1) to give the **4** and **5** as a light yellow solid (43.27 mg, molar ratio of **4** to **5** is about 1:3).

2.5. NMR analysis of compound 1, 2, 3, 4 and 5

^1H and ^{13}C NMR analysis of the purified compounds was carried out to confirm their structure. NMR spectra of compounds 2, 3, 4, 5, 6, 9, 10, 11, 14 and 15 were recorded on a Bruker 400 MHz NMR spectrometer at room temperature in DMSO-d_6. Compound 7 was recorded in $CDCl_3$, with the solvent residual peak as the internal reference. Chemical shifts were expressed in δ values.

2.6. MS analysis of compound 1, 2, 3, 4 and 5

The identities of the purified compounds were confirmed by a Thermo Finnigan LTQ-DECA-XP-MAX linear ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Thermo Finnigan Xcalibur software (version 2.1) was used for data acquisition and processing. The analysis was monitored by an electrospray ionization (ESI) interface with a detector voltage of 1.5 kV, from m/z 150 to 800 in the mass analyzer, and with an even time of 1.0 s. The ESI parameters were as follows: source voltage, 2.50 kV; sheath gas flow rate, 50 arb; aux/sweep gas flow rate, 10 arb; capillary voltage, −41.00 V; and capillary temperature,