

Solid Phase Syntheses of Ferulic Acid Derivatives Acetyl Feruloyl Tyrosine and Acetyl Feruloyl Valyl Tyrosine

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Abstract: Ferulic acid was used as a common drug for cardia-cerebrovascular disease and leukopenia, but the application of ferulic acid was inhibited by the poor absorption and stability. The improvement of these defects can be realized by modifying ferulic acid by amino acids, because the amido bond can increase the bioavailability and therapeutic effect of some drugs based on the peptide transporter system of mammalian which can transport the peptidyl drugs. The peptidyl derivatives of ferulic acid, namely acetyl feruloyl tyrosine and acetyl feruloyl valyl tyrosine, were synthesized using Fmoc solid-phase synthesis method. The synthesized ferulic acid amide derivatives were purified by RP-HPLC, and characterized by IR, ¹H NMR and ESI-MS. The results indicated that Fmoc solid phase synthesis was a convenient method for the amide bond modification of ferulic acid and the further property research on ferulic acid derivatives.

Keywords: Derivative, ferulic acid, purification, solid phase, synthesis, tyrosine, valine.

INTRODUCTION

Ferulic acid, a hydroxycinnamic acid, is an abundant phenolic phytochemical component, and has many medical functions, such as anti-platelet aggregation, inhibiting the generation of platelet thromboxane A₂(TXA₂), anti-inflammatory, analgesic and regulating immune system [1, 2]. Some studies also suggest that ferulic acid is an antioxidant reacting with free radicals and involving DNA damage, cancer and cell aging [3, 4]. For the strong hydrophilicity of ferulic acid, it is difficult to play its roles through the lipid bilayer of biological membrane, but ferulic acid derivatives, such as ferulic acid ester, ferulic acid amide, ferulic acid ether, ferulic acid ketone, aromatic ring substituted ferulic acid, have stronger physiological activity and lower toxicity than ferulic acid [5-8]. In recent years, researches and applications have made great progresses in the modification of drug molecules by amino acids and oligopeptides for improving the stability and absorption of drugs [9, 10], and many reports also showed that the amide derivatives of ferulic acid could promote the insulin secretion, the antioxidation of low density lipoprotein(LDL), the anti-atherosclerosis action and inhibit the cyclooxygenase COX-2 induced inflammatory diseases [11, 12]. Ferulic acid modified by amino acid can possibly improve the stability of ferulic acid, decrease the degradation of ferulic acid, and promote the absorption of ferulic acid through the peptide transporter system in mammalian which can mediate the transport of peptidyl drugs containing amido bond [13].

In this study, the ferulic acid derivatives modified by amino acids, namely acetyl feruloyl tyrosine and acetyl feruloyl valyl tyrosine (F-Tyr and F-Val-Tyr) were efficiently synthesized by Fmoc solid-phase synthesis method and characterized by IR, ¹H NMR and ESI-MS. The advances on the derivatives qualities will be researched and reported in the future.

MATERIALS AND METHODS

Materials

Ferulic acid, piperidine and trifluoroacetic acid (TFA) were purchased from Shanghai Jingchun Reagent Co. Ltd. (Shanghai, China). Fmoc-Tyr(*t*Bu)-Wang Resin (0.38mmol/g), Fmoc-Val-OH, N,N-diisopropylethylamine (DIEA), 1-hydroxybenzotriazole hydrate (HOBT), 2-(1H-Benzotriazole-1-yl)-1,1,3,3-Tetramethyluronium hexafluorophosphate (HBTU) were purchased from GL Biochem Ltd. (Shanghai, China). Dichloromethane (DCM) and acetic anhydride were purchased from Kaifeng Chemical Reagent Factory (Kaifeng, China). DMF, ninhydrin and ether were purchased from Tianjin Kemiou Chemical Reagent Co. Ltd. (Tianjin, China). IR spectrum was obtained on a Shimadzu IR-Prestige21 FTIR meter. ¹H spectroscopy was recorded on a Bruker DPX-400 MHz spectrometer. Mass spectrometry was performed on an Esquire 3000 ESI-MS with an ion trap mass spectrometer (Agilent, USA) working in the positive mode.

Methods

Synthesis of Acetyl Ferulic Acid

A solution containing ferulic acid (1.9421g, 0.01000mol) and NaOH (1.9073g, 0.04578mol) under 10 °C was mixed with 1.2 mL acetic anhydride (1.2790g, 0.01253mol), stirred

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for 10 min at 20 °C and 20 min at room temperature. The pH value of solution was mediated to 4-5 using dilute sulfuric acid. The white precipitate was obtained after filtered and washed with water. Recrystallization was used to obtain colorless flaky crystal (1.685g, 71.4%, m.p.195-199°C) with anhydrous alcohol [14].

Syntheses of F-Tyr and F-Val-Tyr

The syntheses of derivatives were prepared according to the literature [15]. As shown in Scheme 1, the synthesis of F-Val-Tyr was detailed. Fmoc-Tyr(tBu)-Wang Resin (0.1547 g, 0.38 mmol/g) was treated with 20% piperidine in DMF (15 mL/g resin, 3 min), 20% piperidine in DMF (15 mL/g resin, 30 min), DMF (5×15 mL/g resin, 2 min each). At this point, the Kaiser test (5%) was performed to identify the presence of free amine available for coupling. A coupling solution was prepared by adding DIEA (0.145 mL, 0.876 mmol) to a DMF (5 mL) solution containing Fmoc-Val-OH (0.2985 g, 0.880 mmol), HOBt (0.1202 g, 0.890 mmol) and HBTU (0.3366 g, 0.888 mmol) on the ice bath. The coupling solution was added to the peptide synthesis vessel containing the resin and reacted for 1.5 h. After the Kaiser test showed the full coupling, the resin was washed with DMF (10×15 mL/g resin, 2 min each). Then another coupling solution was prepared by adding DIEA (0.145 mL, 0.876 mmol) to a DMF (5 mL) solution containing acetyl ferulic acid (0.2098 g, 0.888 mmol), HOBt (0.1242 g, 0.919 mmol) and HBTU (0.336 g, 0.886 mmol) on the ice bath. The coupling solution was mixed with the resin and reacted for 1.5 h. The resin was then washed with DMF (2×1 mL, 2 min each), DCM (1mL, 2 min each), ether (2×1 mL, 2 min each), and the Kaiser test showed the full coupling. Cleavage of F-Val-Tyr from the resin was accomplished using a solution of 95% TFA, 2.5% thioanisole, 2.5% water. Reaction time for cleavage was 3 h. The crude product was obtained by precipitation in cold diethyl ether.

F-Tyr was obtained from the coupling of acetyl ferulic acid and deprotected Fmoc-Tyr(tBu)-Wang Resin. Purification by HPLC afforded pure F-Tyr and F-Val-Tyr which were light yellow solid.

HPLC Analyses and Purifications of Ferulic Acid Derivatives

Ferulic acid derivatives were analyzed and purified on an Agilent C18 column (9.4 mm × 250 mm) with 5 μm silica as a stationary phase. A gradient elution with eluent A (0.05% TFA in water) and eluent B (0.1% TFA in acetonitrile) (50:50, v/v) was used at a flow rate of 1 mL·min⁻¹. Peaks were detected at 214 nm.

NMR and Mass Measurements

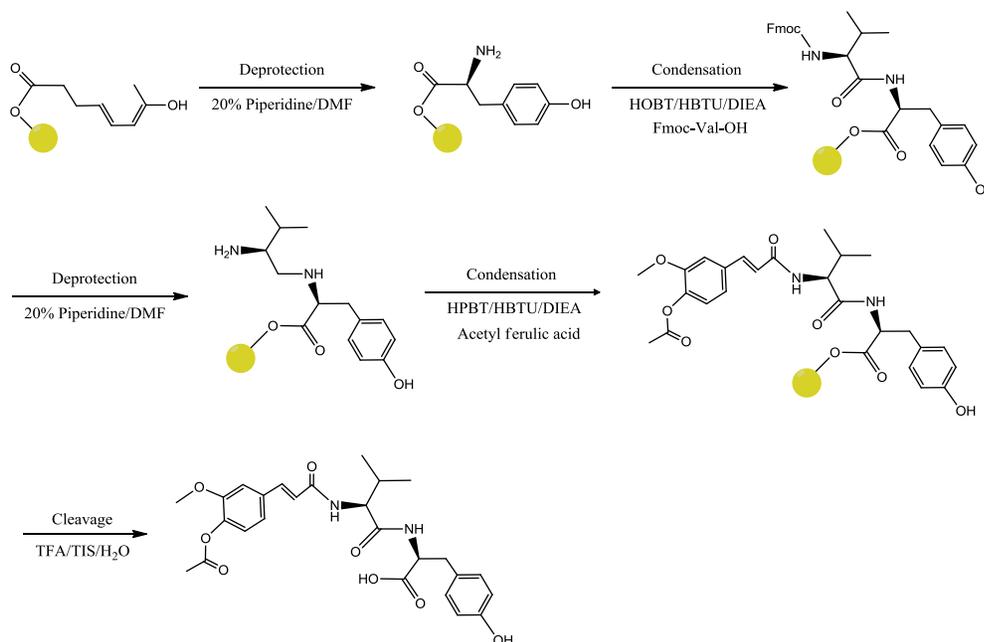
¹H NMR experiments were carried out using a Bruker DPX-400 MHz instrument, using TMS as internal standard. Mass spectra were analyzed on an Agilent Esquire 3000 mass spectrometer fitted with an ion spray source working in positive ion mode, using methanol as solvent.

RESULTS AND DISCUSSION

Syntheses

Acetyl ferulic acid was formed from ferulic acid and acetic anhydride under alkali condition. Acetyl ferulic acid IR (cm⁻¹, KBr Disc): 3071 (ν_{OH}); 3013 (ν_{CH}); 2943, 2847 (ν_{C-H}); 1763 (ν_{C=O}); 1700 (ν_{C=O}); 1632 (ν_{C=C}); 1601, 1506 (ν_{C=C}); 1223 (ν_{C-O-C}) (Fig. 1).

The crude products cleaved from resin were analyzed RP-HPLC, as shown in Fig (2). The peak at 2.523 min retention time in Fig. (2a) and the peak at 3.310 min in Fig. (2b) possibly were corresponded to the target compounds F-Tyr and F-Val-Tyr for larger percentage respectively, which were collected to analyze by ¹H NMR and mass



Scheme 1. Synthesis of F-Val-Tyr.

spectrometry, and were easy to separate from other components for the obviously different retention times. The results of ^1H NMR and mass spectrometry were showed in Fig. (3) and Fig. (4).

The ^1H NMR of F-Tyr (400 MHz, CDCl_3): δ : 7.58 (s, 1H, =CH), 7.00~7.10 (m, 3H, Ar-H), 6.69~6.71 (d, 1H, Ar-H), 6.33~6.37 (s, 2H, Ar-H), 5.0 (s, 1H, -OH), 3.85 (d, 2H, - CH_2), 2.02~2.11 (s, 3H, - CH_3), 3.14~3.19 (m, 3H, - CH_3)

(Fig 3). Mass spectrometry calcd for F-Tyr $\text{C}_{21}\text{H}_{21}\text{NO}_7$: $[\text{M}+\text{H}]^+ = 400.4$. Found: 400.1 and F-Val-Tyr $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}_8$: $[\text{M}+\text{H}]^+ = 499.3$. Found: 498.8 (Fig. 4). These results were according with the modified compounds.

The relative contents of F-Tyr and F-Val-Tyr were determined with area normalization method based on the RP-HPLC profiles (Fig. 2), which indicated ferulic acid derivatives synthesized by solid phase synthesis on wang

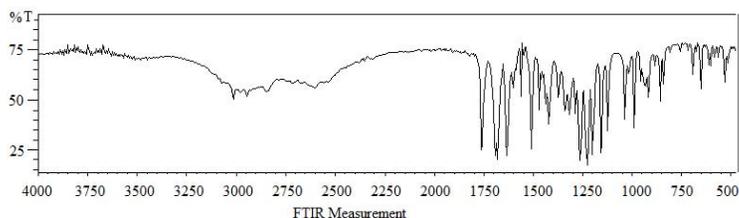


Fig (1). IR spectrum of synthesized acetyl ferulic acid

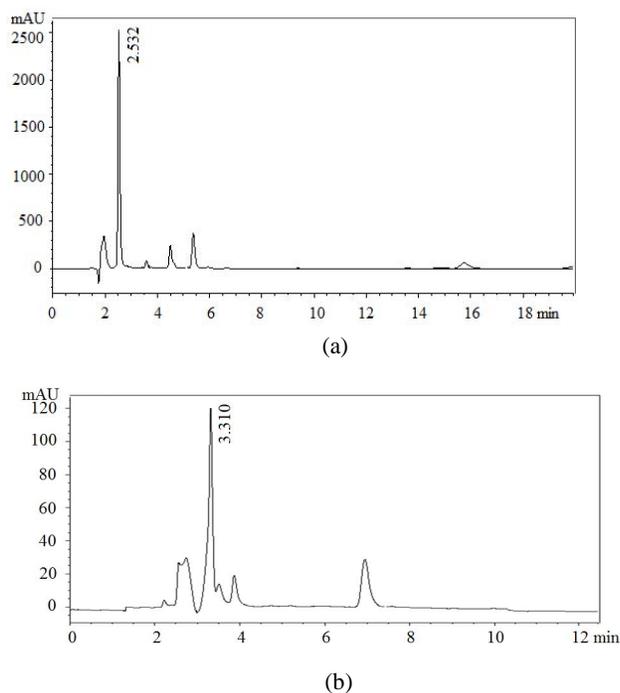


Fig (2). RP-HPLC profiles of F-Tyr (a) and F-Val-Tyr (b) on C18 column.

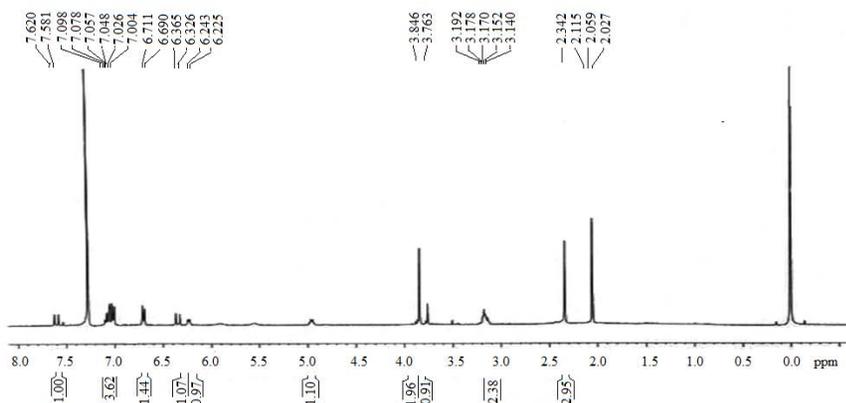


Fig (3). ^1H NMR of F-Tyr.

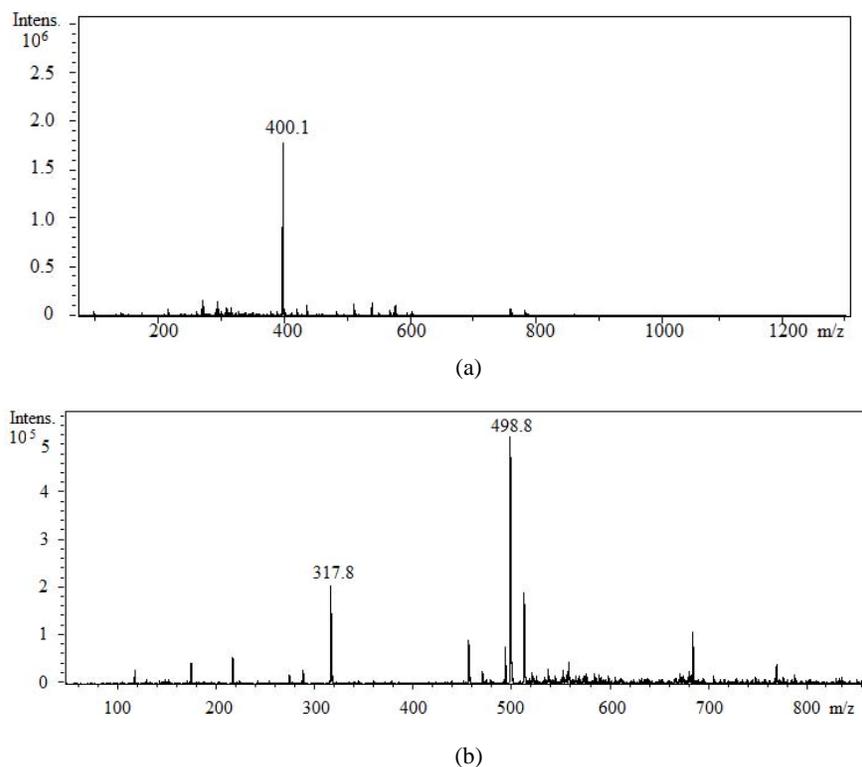


Fig (4). Mass spectra of F-Tyr (a) and F-Val-Tyr (b)

resin with good yield, F-Tyr(85%) and F-Val-Tyr (70%), and also showed that RP-HPLC could be used for further purification.

But many peaks were found in Fig. (4b) except the molecular ion peak (m/z 499.8), and the peak (m/z 317.8) which should be the fragment ion peak for the losing of tyrosine residue, suggesting the synthesis conditions of F-Val-Tyr should be optimized compared with that of F-Tyr.

Fmoc solid phase synthesis is a convenient method for amide bond modifications of drug molecules containing carboxyl and amino, and other drug modifiers also were obtained using the same method in our laboratory [16]. But the conditions of connection, separation and detection in the compound synthesis process still need to be optimized for the different characteristics of components. In conclusion, Fmoc solid phase synthesis method provides a good protection to effectively modify drug molecules and further improve drug properties, and the actual efficacy of modified medicines still need to be studied.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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