Preparation of naringenin fatty acid esters by regioselective acylation

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ABSTRACT

To improve the water solubility without affecting the anti-platelet aggregation activity of naringenin, three acylated derivatives of naringenin, naringenin-7-O-acetate, naringenin-5,7-O-di-propionate and Naringenin-7-O-valerate, were synthesized by a four step synthesis route, benzylation-hydrolysis-acylation-hydrogenation, with naringin as raw material. The yields of naringenin-7-O-acetate, naringenin-5,7-O-di-propionate and Naringenin-7-O-valerate were 87 %, 92 %, 80 %, respectively. The chemical structures of the three derivatives were determined by $^1$H NMR, $^{13}$C NMR and MS.

Key words: Naringenin; Regioselective acylation; Structure identification

INTRODUCTION

Nutriology and Epidemiologic studies show that the intake of flavone with the pathogenesis of cardiovascular was inverse relation[1-3]. It has a immediate correlation about the prevention and cure of cardiovascular diseases which the anti-platelet aggregation activity of naringenin[4-8], therefore, naringenin has a comprehensive prospect in preparing functional food and drugs to prevent and cure the cardiovascular disease. However, naringenin shows poor solubility and stability in most of the solvents, especially in water. This limits the utilization and bioavailability of naringenin. The poor solubility of naringenin partly due to its molecule structure of 2-phenyl-chromone, which prevents the solvent molecules to enter intermolecular of naringenin and solvate the molecule of naringenin. Introducing an aliphatic hydrocarbon side chain into the flavonoid molecule has been proved to be effective to improve its solubility[9,10]. The active groups of naringenin include 4’-OH of B ring and 4 carbonyl of C ring. Though regioselective acylation introducing a short aliphatic chain into 7-OH of naringenin, and keeping the active groups intact is the best method to improve the solubility and keep biological activity[11].

The regioselectivity acylation of flavonoids usually is obtained by enzymic or chemistry-enzymic combined synthesis[12-18], and the traditional chemistry acylation is less adopted. Perhaps it is related to the dilemma that most of hydroxyl groups of flavonoids may be acylated, which leads to a mixture of products with various degree of acylation and the reduction or even lost of the biological activities of flavonoids. However, by now there have been many reports about tradition chemical method to synthesis other flavonoid derivatives, and the route of synthesis, “protection of active group-derivatization-deprotection”, have been reported[19-22]. This route provides valuable reference for synthesis regioselectivity acylation derivatives of flavonoid by tradition chemistry acylation method.

In present paper, to improve the water solubility without affecting the anti-platelet aggregation activity of naringenin, three acylated derivatives of naringenin, naringenin-7-O-acetate (N-ac), naringenin-5,7-O-di-propionate (N-pr) and Naringenin-7-O-valerate (N-va), were synthesized by a four step synthesis route, benzylation-hydrolysis-acylation-hydrogenation, with naringin as raw material. The chemical structures of the three derivatives were determined by $^1$H NMR, $^{13}$C NMR and MS.
EXPERIMENTAL SECTION

Chemicals and Apparatus
Naringin (≥98%) was obtained from Nanjing TCM Institute of Chinese Materia Medica (Nanjing, China). Anhydride and chloride were analytical reagent and purchased from Aladdin Industrial Corporation (Shanghai, China). Benzyl bromide was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), and distilled before used. N, N-dimethylformamide (DMF), triethylamine, and dichloromethane (DCM) were analytical reagent and purchased form Tianjin Kemiu Chemical Reagent Co., Ltd (Tianjin, China), and dehydrated before used. Pd/C (10%) was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Other reaction media and chemicals were purchased from local suppliers.

An Agilent 1200 infinity preparative chromatography system performed chromatographic separation. It was equipped with an Agilent Prep LC controller (Agilent, Santa Clara, USA), a variable wavelength UV detector (VWD-G1314B, Agilent, Santa Clara, USA), a preparative column (ZORBAX-SB C18, 250 × 9.4 mm, 5 µm, Agilent, Santa Clara, USA), a injector (3725i, Agilent, Santa Clara, USA), and an auto-fraction collector (G1364C, Agilent, Santa Clara, USA).

The chemical structures of Naringenin and three acylated derivatives, N-ac, N-pr and N-va, were determined by 1H NMR (600 MHz) and 13C NMR (600 MHz) using a Bruker AC500 spectrometer (Bruker, Courtaboeuf, France) and MS using an Agilent 6410 liquid chromatography-mass spectrometry (Agilent, Santa Clara, USA).

Synthesis of acylated derivatives of naringenin
Naringin as raw material, a complete chemical synthetic procedure, benzylatio-hydrolysis-acylation-hydrogenation, was conducted to obtain three acylated derivatives of naringenin. The synthesis route was expressed in Figure 1.

According to the method described by Wuts,[23] naringin (5.80 g, 0.01 mol) and K₂CO₃ (2.07 g, 0.015 mol) were sequentially added to 50 mL of DMF under nitrogen and stirred for 1 h at room temperature. Then benzyl bromide (1.8 mL, 0.015 mol) was dropped slowly into the reaction mixture. After completion of the addition, the reaction mixture was stirred at 40 °C for 2.5 h under nitrogen and then adjusted to pH 6.0 with 10% (v/v) acetic acid in ice bath. 300 mL deionized water was then added to this mixture and the suspension was filtered. The filter residue was dissolved in 200 mL of 95% (v/v) ethanol at 70 °C. 90 mL of 36% (m/m) hydrochloric acid was added to the solution and the hydrolysis continued at 70 °C for 2 h. The suspension was cooled down to room temperature, and then filtered to give the hydrolyzate. The hydrolyzate was washed with ice water until its pH was neutral.

The hydrolyzate was dissolved in 50 mL of DCM. Acid anhydride (0.015 mol) or acid chloride (0.015mol) and triethylamine (0.015 mol) were added and the mixture was stirred at room temperature until thin-layer chromatography (TLC) analysis showed the complete conversion of the substrate. The reaction mixture was then extracted with 1 mol/L hydrochloric acid and the organic layer was washed with a saturated aqueous solution of NaHCO₃ and deionized water. The pooled extracts were dried over Na₂SO₄ and then taken to dryness under vacuum to give the acylate.

The acylate was dissolved in 500 mL of ethanol/dioxane (1:1, v/v). 1.5 g of Pd/C was added to the solution. The reaction mixture was stirred at room temperature and atmospheric pressure for 6 h under hydrogen, then Pd/C was filtered off and the filtrate was evaporated in vacuo to give the crude product of acylated derivative of naringenin.
TLC analytical process
The acylation process was monitored using TLC on silica gel 60-GF254 (Merck, Darmstadt, Germany), with a solvent mixture of ethyl acetate, methanol and acetic acid (at a ratio of 6:4:0.1, v/v/v). The plate was observed and detected under ultraviolet (UV) light (280 nm).

Purification of acylated derivatives of naringenin
Highly purified acylated derivatives were obtained by semi-preparative high performance liquid chromatography (HPLC). N-ac, N-pr and N-va were purified using the linear elutions of acetonitrile/water (70:30, v/v), (60:40, v/v), (50:50, v/v), respectively. The flow rate was 5 mL/min, and the elution was performed at a room temperature at 280 nm with the UV detector.

Nuclear magnetic resonance (NMR) and mass spectrometry (MS) analytical procedure
The chemical structures of Naringenin and three acylated derivatives, N-ac, N-pr and N-va, were determined by $^1$H NMR (600 MHz) and $^{13}$C NMR (600 MHz) in DMSO-$d_6$ with TMS as an internal reference, and MS without breaking into fragment ions with electrospray ionization (ESI).

RESULTS AND DISCUSSION
Regioselective acylation of naringenin
To ensure that the acylation selectively occurred at hydroxyl group at C7 of naringenin, the strategy, protection of active groups–acylation of hydroxyl group at C7–deprotection groups, was adopted and naringin was chosen as starting material. The synthesis route included four steps.

In the first step, 1.5-fold molar amount of alkaline, anhydrous K$_2$CO$_3$, was added to facilitate the formation of oxygen anions for hydroxyl groups at C4’ of naringenin, while hydroxyl group at C5 kept no conversion due to the formation of intramolecular hydrogen bond with carbonyl at C4 and therefore it was difficult to form oxygen anions [23]. Then, 1.5-fold molar amount of benzyl bromide was added to benzylation the oxygen anions. An aprotic solvent, DMF, was used to avoid the production of C-alkylated derivatives. In the second step, hydroxyl group at C7 was exposed by hydrolysis of the glycoside. In the third step, though both hydroxyl groups at C7 and C5 possibly formed intramolecular hydrogen bond with carbonyl at C4, hydroxyl group at C5 was unreactable compared with C7, because the directionality and saturation of hydrogen bond made it easier to form an intramolecular hydrogen bond with carbonyl at C4 [24]. A slight excess of alkaline, triethylamine, and acyl donors, acid anhydride or acid chloride, that is 1.1-fold molar amount, were added to regioselective ionization and then acylation of hydroxyl group at C7. In the fourth step, the benzyl groups at C4’ was removed by hydrogenation and the crude products of acylated derivatives were obtained.

The crude products were purified using semi-preparative HPLC to afford three highly purified acylated derivatives of naringenin. All of these derivatives were yellow powders. The yields of N-ac, N-pr and N-va were 87 %, 92 %, 80 %, respectively.

Acylated derivatives characterization by $^1$H, $^{13}$C NMR and MS analyses
The number and the position of aliphatic acyl linked to the naringenin were determined by MS spectra and comparing the $^1$H and $^{13}$C NMR spectra between naringenin and three acylated derivatives.

In the $^1$H NMR spectra of the derivatives, most of the active hydrogen of phenolic hydroxyl at C4’ disappeared, and this is the significant feature of 7-substituted naringenin, such as naringin [23]. In the $^{13}$C NMR spectra of the derivatives, the chemical shifts attributed to ester carbonyl carbons of N-ac, N-pr and N-va were observed at 160.23, 170.44, 172.56 ppm, respectively. This suggested the successful introduction of aliphatic acyl into naringenin. In the $^{13}$C NMR spectrum of naringenin, the chemical shift of C7 was at 163.97 ppm, however it was observed to modify to high frequency in $^{13}$C NMR spectra of the derivatives, its chemical shifts in N-ac, N-pr and N-va were at 155.35, 155.90, 155.88 ppm, respectively. Also, the chemical shift of C$_6$ in N-ac, N-pr and N-va were observed at 98.04, 96.89 and 97.07 respectively, which shifted to low frequency comparing with that of naringenin ($\delta$ 96.26 ppm). And the chemical shift of C$_5$ in N-ac, N-pr and N-va were observed at 98.04, 96.89 and 100.14 respectively, which shifted to low frequency comparing with that of naringenin ($\delta$ 95.45 ppm). In addition, C5 of N-pr shift from 163.97 ppm to 150.38ppm. The same chemical shifts of other carbons were observed in the derivatives and naringenin. The above analysis suggested that the acylation was regioselectively occurred at hydroxyl group at C7 of naringenin in N-ac and N-va. However, for N-pr, both C5 and C7 of naringenin were acylated due to the strong active of propionyl chloride. (Fig.1).

The molecular ion peaks in the MS spectra of the derivatives confirmed the molecular structure further. For N-ac, a
peak was observed at 338.34 \text{ m/z }, which was consistent with the summation of molecular weight of N-ac and Na\textsuperscript{+}. Consistently, the peak attributed to the summation of molecular weight of N-pr and Na\textsuperscript{+} was observed at 408.31 \text{ m/z } in the MS spectra of N-pr. And for N-va, a peak was observed at 371.27 which was consistent with the molecular weight of N-va.

\textsuperscript{1}H chemical shifts for N-ac were as follows: \( \delta \) (ppm) 7.005 (2H, H-2', 6'), 6.662 (2H, H-3', 5'), 6.287 (1H, H-6), 6.290 (1H, H-8), 5.472 (1H, H-2), 3.340 (1H, H-3 trans), 3.078 (1H, H-3 cris), 2.171 (\text{CH}_3).

\textsuperscript{13}C chemical shifts for N-ac were as follows: \( \delta \) (ppm) 201.67 (C-4), 170.23 (C=O), 155.35 (C-7), 169.85 (C-5), 169.10 (C-9), 158.20 (C-4'), 131.59 (C-1), 129.53 (C-2',6'), 115.80 (C-3'), 115.51 (C-5'), 102.00 (C-10), 98.04 (C-6), 96.89 (C-8), 77.11 (C-2), 45.74 (C-3), 17.78 (\text{CH}_3).

MS (\text{m/z}) for N-ac: 338.34 [M+Na\textsuperscript{+}].

\textsuperscript{1}H chemical shifts for N-pr were as follows: \( \delta \) (ppm) 7.005 (2H, H-2', 6'), 6.662 (2H, H-3', 5'), 6.453 (1H, H-6), 6.317 (1H, H-8), 5.955 (1H, H-2), 3.046 (1H, H-3 trans), 2.734 (1H, H-3 cris), 2.084 (\text{CH}_2), 0.910 (\text{CH}_3).

\textsuperscript{13}C chemical shifts for N-pr were as follows: \( \delta \) (ppm) 201.67 (C-4), 155.88 (C-7), 170.44 (C=O), 150.38 (C-5), 169.15 (C-9), 158.98 (C-4'), 131.56 (C-1), 129.51 (C-2',C-6'), 116.16 (C-3'), 115.51 (C-5'), 102.65 (C-10), 97.07 (C-6,C-8), 79.88 (C-2), 45.71 (C-3), 29.01 (\text{CH}_2), 10.11 (\text{CH}_3).

MS (\text{m/z}) for N-pr: 408.31 [M+Na\textsuperscript{+}].

\textsuperscript{1}H chemical shifts for N-va were as follows: \( \delta \) (ppm) 7.030 (2H, H-2', 6'), 6.657 (2H, H-3', 5'), 5.103 (1H, H-8), 5.095 (1H, H-6), 5.955 (1H, H-2), 3.369 (1H, H-3 trans), 3.145 (1H, H-3 cris), 2.277 (\text{CH}_2), 1.539 (\text{CH}_3), 1.327 (\text{CH}_2), 0.905 (\text{CH}_3).

\textsuperscript{13}C chemical shifts for N-va were as follows: \( \delta \) (ppm) 201.91 (C-4), 155.90 (C-7), 172.56 (C=O), 171.56 (C-5), 164.21 (C-9), 159.40 (C-4'), 131.58 (C-1), 129.54 (C-2'), 129.42 (C-6'), 115.58 (C-3'), 115.54 (C-5'), 101.85 (C-10), 100.14 (C-6,C-8), 77.21 (C-2), 45.67 (C-3), 33.70 (\text{CH}_2), 27.44 (\text{CH}_2), 22.01 (\text{CH}_2), 14.05 (\text{CH}_3).

MS (\text{m/z}) for N-va: 371.37 [M\textsuperscript{+}].
Fig. 2 $^1$H-NMR spectrum of naringenin-7-O-acetate

Fig. 3 $^{13}$C-NMR spectrum of naringenin-7-O-acetate

Fig. 4 MS spectrum of naringenin-5,7-O-di-propionate
Fig. 5 $^1$H-NMR spectrum of naringenin-5,7-$O$-di-propionate

Fig. 6 $^{13}$C-NMR spectrum of naringenin-5,7-$O$-di-propionate
Fig. 7 MS spectrum of naringenin-7-O-valerate

Fig. 8 $^1$H-NMR spectrum of naringenin-7-O-valerate

Fig. 9 $^{13}$C-NMR spectrum of naringenin-7-O-valerate
CONCLUSION

Three acylated derivatives of Naringenin, N-ac, N-pr and N-va, were synthesized by a four step synthesis route, benzylolation-hydrolysis-acylation-hydrogenation, with naringin as raw material. The yields of N-ac, N-pr and N-va were 87 %, 92 %, 80 %, respectively. The acylation was regioselectively occurred at hydroxyl group at C7 of naringenin for N-ac and N-va and at C5 and C7 for N-pr. The synthesis method realized regioselective acylation of hydroxyl group of naringenin, and gave high yields.

Abbreviations used

N-ac, naringenin-7-O-acetate; N-pr, naringenin-5,7-O-di-propionate; N-va, naringenin-7-O-valerate; NMR, nuclear magnetic resonance; MS, mass spectroscopy; TLC, thin-layer chromatography; ESI, electrospray ionization; UV, ultraviolet; HPLC, high performance liquid chromatography; DMF, N, N-dimethylformamide; DCM, dichloromethane.
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REFERENCES

[10] L Montenegro; C Carbone; C Maniscalco; D Lambusta; G Nicolosi; CA Ventura; G Puglisi. Int. J. Pharm. 2007, 336(2), 257–262.
[16] L Sardone; B Pignatario; F Castelli; MG Sarpietro; G Nicolosi; G Marletta. J Colloid Interface Sci. 2004, 271(2), 329-335.