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THE PREPARATION OF DIOSMIN FROM HESPERIDIN *VIA* THREE STEPS

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Summary

The Preparation of Diosmin from Hesperidin *via* three Steps

Diosmin (diosmetin 7-*O*-rutinoside), the natural flavonoids mainly found in *Citrus* fruits, is used for the treatment of hemorrhoids or chronic venous diseases. In this report, we studied the semi-synthesis of diosmin from hesperidin through three steps. In the first step, hydroxyl functional groups of hesperidin were protected, then acetyl-hesperidin (**1**) was continuously oxidized on the C-3 position, diosmin was obtained by deacetylation reaction. The structure of compounds **1-3** was confirmed by 1D, 2D-NMR, and LC-ESI MS spectrum.

Keywords: *Diosmin, Diosmetin 7-O-rutinoside, Hesperidin, Oxidation, Deacetylated.*

1. Introduction

Chronic venous insufficiency, varicose veins of the lower extremities, and hemorrhoids are quite common diseases today. Chronic venous insufficiency is a condition that occurs when the veins lose their ability to return blood to the heart due to impaired function of the venous valves of the superficial and/or deep venous systems [1]. If tiny valves in these veins are weak or damaged, blood can flow backward and pool in the veins, causing the veins to stretch or twist, that is varicose veins [2]. Same here, the veins are swollen in your anus and lower rectum, called piles [3]. In the world, 300 million people suffering from these diseases of all ages, and the annual cost is about 1 billion USD per year. About 40% of people over 50 years old in Vietnam suffer from it, and 8.2% of these are treated [4]. Many treatments are given such as the use of drugs to treat symptoms, diuretics, and oriental medicine [5]. Several naturally extracted or semi-synthetic flavonoids are like Phlebodia (600 mg diosmin), Flebosmin (300 mg diosmin), Diosmin Arrow (600 mg diosmin) and Daflon (combination of diosmin: hesperidin in a weight ratio of 450 mg: 50 mg) have been used for the treatment of hemorrhoids or chronic venous diseases. They increase flexibility, strengthen, and protect blood vessel walls, reduce capillary permeability, increase venous tone, enhance lymphatic drainage, and inhibit inflammatory mediators [6].

Diosmin is a natural flavonoid mainly present in the peel of some *Citrus* fruits, such as oranges and lemons [7]. Diosmin is a flavonoid that possesses antioxidant and anti-inflammatory properties. Although it has many interesting properties, the content of diosmin in natural plants is very low, and the cost of direct extraction is extremely high, so it is usually prepared by the semi-synthesis from hesperidin which is distributed in the genus *Citrus* is about 6% in *Citrus* fruits [8]. Therefore, the study on the semi-synthesis of diosmin from hesperidin has been of interest to scientists. In the world, many research processes were reported for semi-synthetic diosmin from hesperidin at the laboratory scale [9-12]. According to Cremades, diosmin was synthesized by using *N*-bromosuccinimide for the bromination of acetyl hesperidin in the presence of benzoyl peroxide in chloroform and obtained 44% yield [13]. In another report, hesperidin was treated with aqueous sodium hydroxide in the presence of iodine and pyridine at 100°C to obtain diosmin with a yield of 66% [10]. In this study, we reported a process for the preparation of diosmin from hesperidin. The process involves the

oxidation of acetylated hesperidin (**1**) with NaI in pyridine in the C-3 position and then deacetylated to obtain diosmin (**3**).

2. Materials and methods

2.1. Materials

Hesperidin, pyridine, $(CH_3CO)_2O$ were commercially purchased from Sigma -Aldrich (St. Louis, MO, USA) or TCI (Tokyo, Japan). If necessary, solvents were purified and or dried to be used. Analytical thin layer chromatography (TLC) was performed on pre-coated *silica gel* 60 F_{254} plates (Merck) and observed under 254 nm and 360 nm. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM 500 FT-NMR instrument in DMSO-d_6 solvent, chemical shifts (δ) are in ppm relative to tetramethylsilane (TMS), and coupling constants (J) are in Hz. LC-ESI MS coupled mass spectrometry was recorded on an LC/MS-8045 Shimadzu.

2.2. Methods

Diosmin was prepared three steps away from hesperidin. The first step of the process was the acetylation of OH sites of the sugar rings, 3′-OH and 5′-OH groups of hesperidin. Following a single bond oxidation reaction at the C-3 position using the oxidizing reagent NaI in DMSO gave acetyl-diosmin (**2**). Deacetylation of acetyl-diosmin (**2**) in 5% NaOH afforded diosmin (**3**).

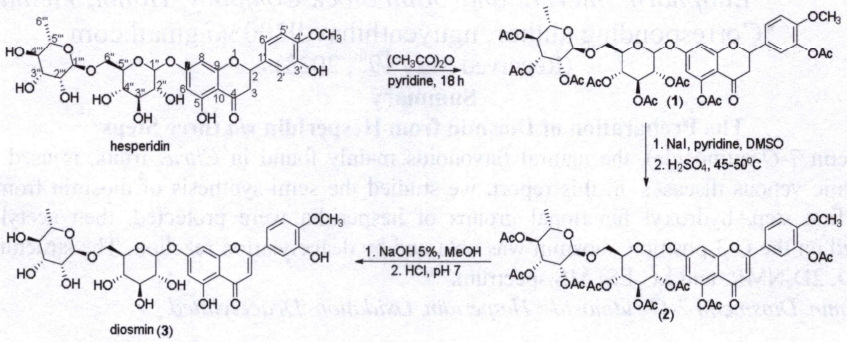

Fig. 1. Synthesis of diosmin (**3**) from hesperidin.

3. Results and discussion

3.1. Synthesis of acetyl-hesperidin (1)

Hesperidin (0.1 g; 0.164 mmol, 1 equiv.) was dissolved in pyridine (1 mL) and added acetic anhydride (0.195 mL; 1.968 mmol; 12.5 equiv.). The reaction was stirred at room temperature for 18 h, monitored by thin layer chromatography with solvent system *n*-hexane: acetone = 1:1 (v/v). The precipitated product was obtained by adding water to the reaction mixture, filtered, and washed with water to remove the pyridine to afford a solid as acetyl-hesperidin (**1**) (0.145 g). Yield: 95%. Melting point: 150-153°C. LC-ESI MS: $[M-H]^-$ *m/z* 945.35. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H (ppm): 1.01 (3H, d, J = 6.0 Hz, H-6‴), 1.92-2.06 (18H, s, 6 x $COCH_3$), 2.26 (6H, s, 3′-$COCH_3$, 5-$COCH_3$), 2.66 (1H, ddd, J = 2.8; 11.6; 16.6 Hz, H-3b), 3.19 (1H, ddd, J = 7.6; 13.0; 16.6 Hz, H-3a), 3.39 (1H, m, H-5‴), 3.60 (1H, m, H-6″a), 3.73 (1H, d, J = 9.8 Hz, H-6″b), 3.79 (3H, s, 4′-OCH_3), 4.25 (1H, m, H-5″), 4.73 (1H, s, H-1‴), 4.82 (1H, t, J = 10.0 Hz, H-4‴), 5.03-5.05 (3H, m, H-4″, 2″, 3‴), 5.09 (1H, m, H-2‴), 5.33 (1H, m, H-3″), 5.55 (1H, ddd, J = 2.8; 6.0; 13.2 Hz, H-2), 5.76 (1H, dd, J = 3.6; 7.8 Hz, H-1″), 6.45 (1H, q, J = 2.2; 3.4 Hz, H-8), 6.68 (1H, dd, J = 2.2, 18.4 Hz, H-6), 7.16 (1H, d, J = 8.6 Hz, H-5′), 7.28 (1H, dd, J = 2.0; 8.6 Hz, H-2′), 7.39 (1H, td, J = 2.0, 8.6 Hz, H-6′). ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C (ppm): 17.0 (C-6‴), 20.3-20.8 (8 x $COCH_3$), 43.6 (C-3), 56.0 (4′-OCH_3), 65.2 (C-6″), 65.9 (C-5‴), 68.1 (C-3‴), 68.6 (C-4″), 68.7 (C-2‴), 70.0 (C-4‴), 70.5 (C-2″), 71.9 (C-3″), 72.1 (C-5″), 78.2 (C-2), 95.9 (C-1″), 96.9 (C-1‴), 101.7 (C-6), 105.3 (C-8), 109.1 (C-10), 112.7 (C-5′), 121.5 (C-2′), 125.7 (C-6′), 130.8 (C-1′), 139.2 (C-3′), 151.2 (C-9), 151.5 (C-4′), 161.4 (C-7), 163.4 (C-5), 168.5-169.7 (8 x $COCH_3$), 188.7 (C-4).

3.2. Synthesis of acetyl-diosmin (2)

The mixture of acetyl-hesperidin (**1**) (0.1 g; 0.11 mmol; 1 equiv.) and NaI (0.01 g; 0.67 mmol; 0.6 equiv) was dissolved in a mixture of DMSO (0.5 mL) and pyridine (0.5 ml). After the solid mixture was completely dissolved, the reaction mixture was slowly added concentrated H_2SO_4 to reach *pH* 5 and carried out at a temperature of 55-60°C. The reaction was monitored by thin-layer chromatography with solvent system *n*-hexane: acetone = 1:1 (v/v). The reaction was extracted with ethyl acetate solvent (3 x 40 ml), and the organic phase was dried with anhydrous Na_2SO_4, filtered, and recovered under reduced pressure. The crude product was purified by normal phase column

chromatography with the *n*-hexane: acetone system from 6:1 to 3:1 (v/v) to give acetyl-diosmin (**2**) (0.09 g). Yield 90%. Melting point: 145-148°C. LC-ESI MS: $[M+H]^+$ 945.30. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H (ppm): 0.99 (3H, d, J = 6.2 Hz, H-6‴), 1.86 (3H, s, $COCH_3$), 1.97-2.04 (15H, s, 5 x $COCH_3$), 2.30 (3H, s, 3′-$COCH_3$), 3,45 (1H, m, H-6″a), 3.64 (1H, dd, J = 5.1; 11.9 Hz, H-6″b), 3.77 (1H, m, H-5‴), 3.88 (3H, s, 4′-OCH_3), 4.31 (1H, m, H-2″), 4.75 (1H, d, J = 1.4 Hz, H-1‴), 4.81 (1H, t, J = 10.0 Hz, H-2‴), 5.01-5.11 (5H, m, H-4‴, 5″, 3″, 3‴), 5.38 (1H, t, J = 9.6 Hz, H-4″), 5.90 (1H, d, J = 8.0 Hz, H-1″), 6.78 (1H, s, H-3), 6.84 (1H, d, J = 2.4 Hz, H-8), 7.31 (1H, d, J = 9.0 Hz, H-6), 7.36 (1H, d, J = 2.4 Hz, H-5′), 7.84 (1H, d, J = 2.4 Hz, H-2′), 7.97 (1H, dd, J = 2.4; 8.6 Hz, H-6′). ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C (ppm): 17.0 (C-6‴), 20.2-20.4 (7 x $COCH_3$), 20.8 ($COCH_3$), 56.0 (4′-OCH_3), 65.3 (C-6″), 65.9 (C-5‴), 68.1 (C-4‴), 68.5 (C-3″), 68.7 (C-3‴), 69.7 (C-2‴), 70.5 (C-4″), 71.9 (C-2″), 72.3 (C-5″), 96.2 (C-1″), 96.9 (C-1‴), 102.4 (C-6), 106.8 (C-3), 108.7 (C-8), 112.0 (C-10), 113.2 (C-5′), 121.0 (C-2′), 123.0 (C-6′), 125.6 (C-1′), 139.6 (C-3′), 150.1 (C-9′), 153.9 (C-4′), 157.8 (C-7), 159.5 (C-5), 160.8 (C-2), 175.2 (C-4).

3.3. Synthesis of diosmin (3)

Acetyl-diosmin (**2**) (0.1 g) was dissolved in 10 mL of MeOH, further 0.5 mL of NaOH 5% was added to the solution mixture. The reaction was refluxed for 15 minutes. The reaction medium was then neutralized with HCl 0.5% solution to *pH* 7. A yellow precipitate was formed, stirred vigorously, then allowed the solution to precipitate completely, filtered, and washed with water to obtain diosmin (**3**) (0.064 g). Yield 95%. Melting point: 277-280°C. LC-ESI MS: $[M-H]^-$ *m/z* 607.40. ^{1}H NMR (DMSO-d_6, 500 MHz) δ_H (ppm): 1.08 (3H, d, J = 6.0 Hz, H-6‴), 3.21 (2H, m, H-4‴, 5‴), 3.33-3.52 (5H, m, H-3‴, 4″, 6″, 5″, 3″), 3.66 (1H, t, J = 8.0 Hz , H-2‴), 3.72 (1H, s, H-2″), 3.87 (3H, s, 4′-OCH_3), 4.61 (1H, s, H-1‴), 5.49 (1H, s, OH), 5.26 (2H, s, 2xOH), 5.12 (1H, d, J = 7.0 Hz, H-1″), 6.46 (1H, d, J = 1.6 Hz, H-6), 6.76 (1H, d, J = 1.4 Hz, H-3), 6.80 (1H, s, H-8), 7.13 (1H, d, J = 8.6 Hz, H-5′), 7.44 (1H, d, J = 2.0 Hz, H-2′), 7.56 (1H, d, J = 8.6 Hz, H-6′). ^{13}C NMR (DMSO-d_6, 125 MHz) δ_C (ppm): 17.8 (C-6‴), 55.8 (4′-OCH_3), 66.1 (C-6″), 68.3 (C-5‴), 69.6 (C-3″), 70.3 (C-4‴), 70.7 (C-3‴), 72.1 (C-2‴), 73.1 (C-4″), 75.6 (C-2″), 76.3 (C-5″), 94.8 (C-1″), 99.6 (C-6), 100.0 (C-8), 100.5 (C-1‴), 103.8 (C-3), 105.5 (C-10), 112.3 (C-5′), 113.1 (C-2′), 118.9 (C-6′), 112.9 (C-1′), 146.8 (C-3′), 151.4 (C-9), 157.0 (C-4′), 161.2 (C-7), 163.0 (C-5), 164.2 (C-2), 182.0 (C-4) [12].

3.4. Discussion

Firstly, the acetylation of hesperidin at the positions of the hydroxyl (OH) groups with acetylation reagent $(CH_3CO)_2O$ in pyridine was obtained without isolation of the acylated hesperidin. All OH groups of rutinose rings and two other OH groups of flavonoid were acetylated to give acetyl-hesperidin (**1**). In ^{1}H NMR spectrum showed eight acetyl groups at δ_H 1.92-2.06 (18H, s, 6 x $COCH_3$) and 2.26 (6H, s, 3′-$COCH_3$, 5-$COCH_3$). In addition, the HMBC spectrum displayed the correlation between the proton of methyl groups at δ_H 2.26 (3H, s, 3′-$COCH_3$) and carbon peak at δ_C 139.2 (C-3′).

Following, the single bond oxidation at the C-3 of acetyl-hesperidin using an oxidation reagent was NaI in DMSO at 55-60°C to obtain a yield up to 90%. Similarly, Rajiv Sakhardande synthesised acetyl-diosmin (2) by iodination of hesperidin followed by elimination of HI, acetyl-diosmin (2) was purified by base acid treatment with an overall reported yield of 60% [10]. Meanwhile, Nguyen Thi My Duyen prepared acetyl-diosmin (2) by bromination and debromination of acetyl-hesperidin (1) in the presence of *N*-bromosuccinimide (NBS) and $CHCl_3$ at 75°C and 6 h to afford acetyl-diosmin (2) up to 54.6%. [12] This is the most important part of the diosmin synthesis process. The acylated hesperidin was oxidized to acylated diosmin through a halogenation and dehydrohalogenation mechanism. In particular, a halogen atom (I) was first added to the C-3 position of acetyl-hesperidin, and subsequently, a double bond was formed by releasing hydrogen halide (HI). Therefore, the oxidation reaction from single bond to double bond creates I_2 which is preferably used in 0.6 molar equivalent of NaI amount relative to acylated hesperidin, and the amount of acid produced by the reaction is stabilized by pyridine. The reaction mixture was extracted with organic solvent and given acetyl-diosmin (**2**) that spectrum is similar to acetyl-hesperidin (**1**) except for the presence of a double bond proton at the C-3 position (δ_H 6.78 (1H, s, H-3). Moreover, in ^{13}CNMR spectrum indicated the signal of carbon double bond peaks at δ_C 160.8, 106.8 which were assigned to the C-2 and C-3 positions of the flavonoid framework.

Finally, hydrolysis of the protect group acetyl-

diosmin (**2**) in methanol, NaOH 5%, the *pH* of the reaction medium is preferably greater than 11, the reaction is refluxed for 15 minutes and diosmin can be subsequently isolated by adding HCl 0.5% to the reaction medium, so diosmin is precipitated and can be recovered by filtration to obtained diosmin (**3**), give the yield up to 95% and HPLC's purity 98.6%. According to Nguyen Thi My Duyen, diosmin (**3**) was synthesized from hesperidin through three steps. In the first step, some functional groups of hesperidin were protected, then it continuously suffered a process of halogenation on position C-3, and finally, it was dehalogenated to get diosmin which contents of 88.98% in HPLC [12]. Furthermore, Nguyen Van Thanh, diosmin was also synthesized *via* the oxidation of hesperidin with iodine in ion liquid including [BMIM]Br, [BMIM]BF4, [BMIM]OTf, [BPY]Br, [BPY]BF4 and [BPY]OTf. As a result, the process allows for obtaining diosmin with high yields of 79-85% [9].

4. Conclusion

In this study, diosmin (**3**) was synthesized through three steps with an efficiency per step of 90% and the overall yield of the process was 82.1%. Our synthesis is a suitable procedure for the semi-synthesis of diosmin with high yield at the laboratory scale. This is the initial result in the semi-synthesis of diosmin. It is necessary to study on process optimization to limit the use of some toxic solvents such as pyridine and MeOH, before scaling it up to the pilot scale.

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QUANTITATIVE DETERMINATION OF AGNUSIDE IN THE FRUIT OF *VITEX TRIFOLIA* L. COLLECTED IN VIETNAM

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Summary

Quantitative Determination of Agnuside in the Fruit of *Vitex trifolia* L. Collected in Vietnam

Agnuside is the main bioactive compound in *Vitex* species. A simple and sensitive high performance liquid chromatography (HPLC-UV) method was developed for the identification and quantification of agnuside in the fruit of *Vitex trifolia* L. The chromatography was achieved on a Cosmosil 5C18-AR-II (250 mm x 4.6 mm, 5 µm) column at 25°C, using acetonitrile and *O*-phosphoric acid - water (0.4%, v/v) as the mobile phases in the gradient elution mode. The flow rate of 1.4 ml/min and detection wavelength of 258 nm were selected. The developed method was validated as per the ICH guidelines for limit of detection, limit of quantification, linearity, accuracy, and precision for the quantitative analysis of agnuside in 22 fruit samples of *Vitex trifolia* L. The contents of agnuside ranged from 0.12 to 0.38%.

Keywords: *Vitex trifolia* L., *Agnuside, HPLC.*