Short Note

(E)-6-Hydroxy-2-Oxo-2H-Chromen-7-Yl 3-(4-Hydroxy-3-Methoxyphenyl)acrylate

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(*E*)-6-Hydroxy-2-Oxo-2*H*-Chromen-7-yl 3-(4-Hydroxy-3-Methoxyphenyl)Acrylate

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Abstract: A conjugate compound **5**, (*E*)-6-hydroxy-2-oxo-2*H*-chromen-7-yl 3-(4-hydroxy-3-methoxyphenyl)acrylate, of 6,7-hydroxycoumarin (esculetin) **3** and (*E*)-3-(4-hydroxy-3-methoxyphenyl)- acrylic acid (ferulic acid) **1** was prepared in 61% yield by the esterification reaction of a protected ferulic acid **2a** with esculetin **3** in the presence of triethylamine in dichloromethane for 3 h, followed by deprotection using 3M HCl. The structure of compound **5** was confirmed by ^{1}H, ^{13}C NMR spectroscopy, mass-spectrometry and elemental analysis.

Keywords: coumarin; esculetin; ferulic acid; esterification; antioxidant

1. Introduction

Many coumarin-based derivatives are important structural scaffolds for the synthesis of potential biologically active compounds with different pharmacological applications [1]. They continue to be designed and synthesized [2] because of remarkable biological properties, including anticancer [3], anticonvulsant [4] antimicrobial [5], and antiviral [6] activities. Coumarins having intramolecular charge transfer character have also been investigated for fluorescence sensors [7,8]. Among them, 6,7-dihydroxycoumarin (esculetin) **3** displayed various biological activities such as anticancer [9,10], free radical scavenging [11], anti-inflammatory [12], anti-arthritic [13], and hepatoprotective [14]. And, (*E*)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid (ferulic acid) **1** is widely present in seeds, nuts, leaves, and fruits. It has many pharmacological effects including antioxidant [15], anticancer [16], neuroprotective [17], and anti-metabolic syndrome [18]. However, there have been no reports on the synthesis of hybrid compounds made of **1** and **3**. We report herein the synthesis of a new hybrid compound **5** of potential biological interest, (*E*)-6-hydroxy-2-oxo-2*H*-chromen-7-yl 3-(4-hydroxy-3-methoxyphenyl)acrylate.

2. Results

The new compound **5** was prepared as shown in Scheme 1. The hydroxy group of starting material **1** was first protected with acetic anhydride and pyridine according to the previously reported procedure [19] to give **2**, (*E*)-3-(4-acetoxy-3-methoxyphenyl)acrylic acid. After **2** was activated with oxalyl chloride including DMF, the resultant **2a** was allowed to react with **3** in dichloromethane at room temperature for 3 h in the presence of triethylamine to afford an esterified product **4**, (*E*)-6-hydroxy-2-oxo-2*H*-chromen-7-yl 3-(4-acetoxy-3-methoxyphenyl)acrylate in 72% yield. The deprotection of acetyl group of **4** was achieved by use of 3M HCl solution in acetone at room temperature for 24 h to give a conjugate compound **5** in 85% yield.

The ^{1}H NMR spectrum of **4** showed the expected pattern with two sharp singlets at δ 3.82 and 2.24 ppm attributed to methoxy and acetyl protons, respectively, and two doublets at δ 7.81 and 6.93 ppm (J = 16.0 Hz) due to *trans* vinyl protons in ferulic acid moiety. It also showed two doublets at δ 7.89 and 6.24 ppm (J = 9.5 Hz) due to *cis* vinyl protons of esculetin moiety, and the aromatic protons were shown as two singlets at δ 7.48, 6.86 and three doublets at δ 7.59 (d, J = 1.7 Hz), 7.36 (dd, J = 8.2, 1.7 Hz) and 7.13 ppm (d, J = 8.2 Hz). A sharp singlet at δ 10.93 of low field was shown for a hydroxy

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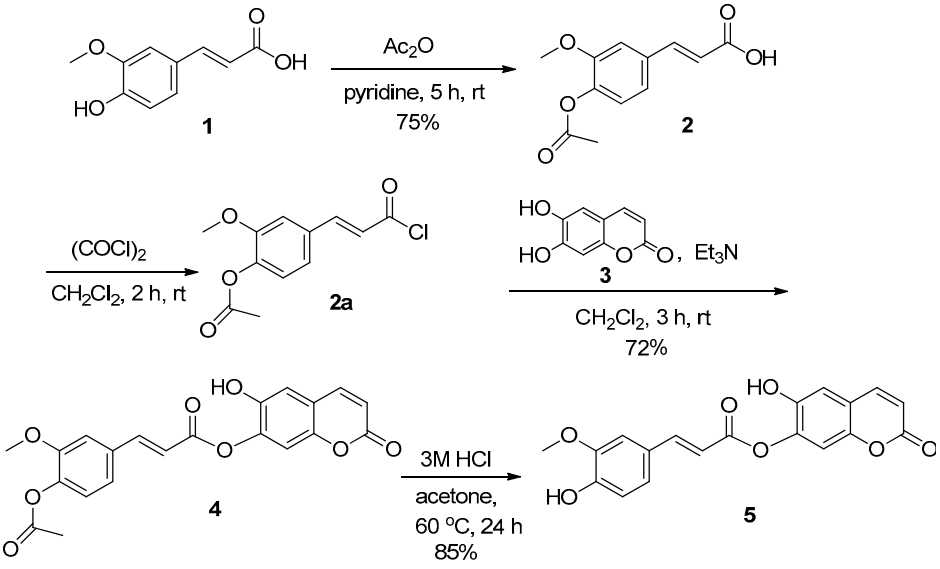
proton of esculetin moiety (Supplementary Materials). In the ^{13}C NMR spectrum, compound **4** displayed three peaks δ 168.8, 165.0, 160.7 ppm for the two carbonyl and newly formed an ester carbon, including sixteen peaks for aromatic and vinyl carbons at δ 153.6, 153.2, 151.7, 146.4, 144.5, 141.9, 136.1, 133.3, 123.8, 122.5 (2C), 117.7, 112.9, 112.8, 111.4, 104.0 ppm, and two peaks for two methyl carbons at δ 56.6, 20.9 ppm. The mass spectrum showed *m*/*z* = 395 (M^+-1) corresponding the molecular formula, $C_{21}H_{16}O_8$, and elemental analysis also provided satisfactory results.



Scheme 1. Synthesis of the target compound **5**.

Compound **5** was confirmed by the absence of signals such as acetyl protons at δ 2.24 ppm in the ^1H NMR, and a carbonyl carbon at δ 168.8 ppm in the ^{13}C NMR spectrum, compared to the spectra of compound **4**. Two singlets due to two hydroxy groups were shown at δ 10.88 and 9.64 ppm in the ^1H NMR spectrum. The mass spectrum provided *m*/*z* = 353 (M^+-1) corresponding the molecular formula, $C_{19}H_{14}O_7$, and elemental analysis gave satisfactory results. The preliminary biological test of DPPH free radical scavenging activity [20,21] for **4** and **5** as antioxidant exhibited SC_{50} values of 40.4 and 2.36 μg/mL, respectively, compared to **1** (2.58 μg/mL) and **3** (0.82 μg/mL) with ascorbic acid (1.65 μg/mL) as positive control.

In conclusion, a new compound **5** of biological importance was effectively prepared in 61% yield by the esterification reaction of a protected ferulic acid **2a** with esculetin **3** in the presence of triethylamine in dichloromethane for 3 h, followed by deprotection of acetyl group using 3M HCl in acetone.

3. Materials and Methods

3.1. General Information

Ferulic acid, esculetin, oxalic chloride, acetic anhydride, triethylamine, 1,1-diphenyl-2-picryhydrazyl (DPPH), ascorbic acid, and the dry organic solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and TCI (Tokyo, Japan). Melting point was determined on Kofler apparatus. Thin-layer chromatography (TLC) was used to monitor reactions and performed using aluminum sheets precoated with silica gel 60 (HF_{254}, Merck, Waltham, MA, USA), and visualized with UV radiation (Fisher Scientific, Waltham, MA, USA). The ^1H, and ^{13}C NMR spectrum were recorded in deuterated DMSO with TMS as the standard on a JEOL JNM-ECZ600R 500 FT-NMR (Tokyo, Japan). The mass spectrum was obtained with AGILENT1100 LCMS (Santa Clara, CA, USA) under electrospray ionization (ESI) conditions. Absorbance for the compounds was measured using SpectraMax Paradigm multi-mode microplate reader (San Jose, CA, USA).

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*3.2. Synthesis of (E)-6-Hydroxy-2-Oxo-2H-Chromen-7-yl 3-(4-Acetoxy-3-Methoxyphenyl)Acrylate (**4**)*

To a stirred solution of **2** (1.0 g, 4.23 mmol) in dry dichloromethane (20 mL) containing few drops of DMF was added oxalyl chloride (1.07 g, 8.45 mmol), and stirred at room temperature for 2 h. After evaporation of the solution, the mixture was diluted with dichloromethane (20 mL), and added **3** (0.75 g, 4.23 mmol) and triethylamine (1.19 mL, 8.50 mmol). The resulting solution was stirred at room temperature for 3 h with monitoring. When the reaction was completed, the mixture was washed with 0.1M HCl solution (10 mL), water (10 mL), and extracted with dichloromethane (2 x 15 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by column chromatography (eluent: ethyl acetate/*n*-hexane = 1/1, v/v) and recrystallized from ethanol to give white solid of **4** in 72% yield (1.20 g). Mp 212–213 °C; TLC *Rf* = 0.48 (dichloromethane/MeOH = 90/10). ^{1}H NMR (500 MHz, DMSO-d_6) (ppm) δ 10.93 (s, 1H), 7.89 (d, *J* = 9.5 Hz, 1H), 7.81 (d, *J* = 16.0 Hz, 1H), 7.59 (d, *J* = 1.7 Hz, 1H), 7.48 (s, 1H), 7.36 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 16.0 Hz, 1H), 6.86 (s, 1H), 6.24 (d, *J* = 9.5 Hz, 1H), 3.81 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) (ppm) δ 168.8, 165.0, 160.7, 153.6, 153.2, 151.7, 146.4, 144.5, 141.9, 136.1, 133.3, 123.8, 122.5 (2C), 116.1, 113.6, 112.8, 112.1, 111.3, 104.0, 56.3. MS (ESI) *m/z* = 395 (M^+-1). Anal. calcd. for $C_{21}H_{16}O_8$, %: C, 63.64; H, 4.07. Found, %: C, 63.88; H, 4.20.

*3.3. Synthesis of (E)-6-Hydroxy-2-Oxo-2H-Chromen-7-yl 3-(4-Hydroxy-3-Methoxyphenyl)Acrylate (**5**)*

A solution of **4** (1.0 g, 2.82 mmol) in acetone (15 mL) containing 3M HCl (1 mL) was heated at 60 °C with stirring for 24 h. After reaction was completed, the mixture was added to saturated aqueous sodium bicarbonate (10 mL) and extracted with ethyl acetate (2 x 15 mL). The organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: dichloromethane/MeOH = 95/5, v/v) and recrystallized from ethanol to give white solid of **5** in 85% yield (0.84 g). Mp 232–233 °C; TLC *Rf* = 0.38 (dichloromethane/MeOH = 90/10). ^{1}H NMR (500 MHz, DMSO-d_6) (ppm) δ 10.88 (s, 1H), 9.64 (s, 1H), 7.88 (d, *J* = 9.5 Hz, 1H), 7.71 (d, *J* = 15.9 Hz, 1H), 7.45 (s, 1H), 7.39 (d, *J* = 1.6 Hz, 1H), 7.18 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.85 (s, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.69 (d, *J* = 15.9 Hz, 1H), 6.24 (d, *J* = 9.5 Hz, 1H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) (ppm) δ 165.3, 160.7, 153.7, 153.1, 150.3, 148.5, 147.6, 144.5, 136.3, 125.9, 124.1, 122.5, 116.1, 113.6, 112.8, 112.1, 111.3, 104.0, 56.6. MS (ESI) *m/z* = 353 (M^+-1). Anal. calcd. for $C_{19}H_{14}O_7$, %: C, 64.41; H, 3.98. Found, %: C, 64.30; H, 4.09. *3.4. DPPH radical scavenging assay for the compounds.*

Each sample was dissolved in methanol at various concentrations ranging from 0 to 100 μg/mL. Then, 50 μL of the sample solution was mixed with 450 μL of a DPPH solution (400 μM) and incubated for 30 minutes at 4 °C. The absorbance was measured at 517 nm using a microplate reader (SpectraMax Paradigm). The SC_{50}, which is the minimum concentration (μg/mL) required to scavenge 50% of the DPPH radicals, was calculated based on the measured absorbance. Ascorbic acid was used as a positive control.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1-S6: ^{1}H NMR, ^{13}C NMR, and Mass spectra of compound **4** and **5**.

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