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| **Authors** | Yu Sasaki, Yuto Nishidono, Ken Tanaka and Tohru Kamitanaka |
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| **ORCID® iDs** | Tohru Kamitanaka - https://orcid.org/0000-0002-2386-6574 |

# Synthesis and Structural Identification of Taxifolin Sulfate Conjugates

Yu Sasaki, Yuto Nishidono, Ken Tanaka, Tohru Kamitanaka*

Address: College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu 525-8577, Shiga, Japan

Email: Tohru Kamitanaka* – t-kmtnk@fc.ritsumei.ac.jp

* Corresponding author

## Abstract

Taxifolin is a naturally occurring flavonoid widely found in plants and foods. It possesses various biological activities and exhibits low toxicity, making it a promising candidate for the prevention and treatment of various diseases. Moreover, taxifolin metabolites particularly sulfate conjugates are gaining attention as biologically significant compounds. Although taxifolin metabolites can be comprehensively detected, their chemical structures remain unclear. Therefore, in this study, simple synthesis methods are established for these compounds further advance the research on taxifolin sulfate conjugates. Four taxifolin sulfate conjugates were synthesized using taxifolin as the starting material. Taxifolin 3-*O*-sulfate and 7-*O*-sulfate were obtained via a stepwise synthesis employing protective groups, and 3′-*O*-sulfate and 4′-*O*-sulfate were directly synthesized from taxifolin and isolated using high-performance

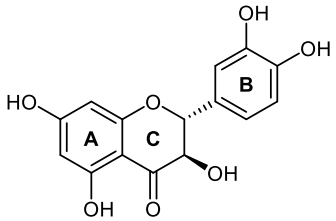
liquid chromatography. The chemical structures of the sulfate conjugates were subsequently elucidated using NMR and mass spectroscopy.


## Keywords

metabolites, sulfate conjugates, taxifolin


## Introduction

Taxifolin is a flavonoid widely found in plants, with particularly high concentrations present in conifers, such as *Pinus roxburghii* and *Cedrus deodara* [1]. It is also present in herbs such as milk thistle (*Silybum marianum*), and folk medicines such as *Persicaria orientalis*, and everyday foods such as olive oil and onions [2-4]. Moreover, taxifolin is also included in dietary supplements because of its strong antioxidant properties. Thus, it is a ubiquitous biologically active compound that is regularly consumed by many people.




**Scheme 1:** Structure of taxifolin.

Its strong antioxidant activity is mainly attributed to the catechol structure in the B ring, which provides superior free-radical scavenging ability compared to many other antioxidant compounds [5]. Numerous *in vivo* and *in vitro* studies have demonstrated the potential of taxifolin in the treatment and prevention of various diseases, including

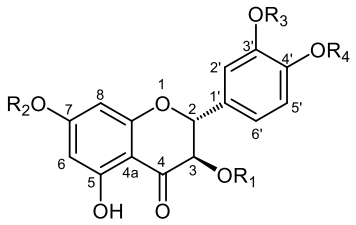
Alzheimer's disease, cancer, cardiovascular diseases, and diabetes [6-8]. Owing to its diverse biological activities and relatively low toxicity compared with other flavonoids [9], taxifolin has attracted considerable pharmacological interest as a promising preventive and therapeutic agent for a broad spectrum of diseases.

It has been recently suggested that the pharmacological activity of biologically active compounds may be mediated not only by the parent compound but also by their metabolites, such as sulfate conjugates. For example, sulfate conjugates of flavonoids have demonstrated various biological activities, such as antioxidant, anticoagulant, and procoagulant properties, highlighting their potential for drug discovery [10-13]. Therefore, flavonoid sulfate conjugates are gaining recognition not only as excretory metabolites, but also as biologically active molecules with considerable promise as safe and effective therapeutic agents [14,15].

A detailed study of taxifolin metabolites in human and rat hepatocytes reported that sulfate conjugates were the most prevalent metabolites [16,17]. A comprehensive analysis identified 191 metabolites, including nine monosulfate conjugate isomers, in rat blood, faeces, urine, and liver [18]. In addition, time-dependent changes in the tissue distribution and excretion concentrations of several taxifolin metabolites in rats have been reported [19]. In humans, taxifolin sulfate conjugates have also been reported as metabolites of silymarin, a flavonolignan complex extracted from milk thistle [20]. However, in these reports, the sulfate conjugates were identified only thorough LC–MS analysis, and further work toward their isolation and structural characterization is still lacking.

The only reported synthesis of taxifolin monosulfate conjugates involves the formation of a mixture of 3′- and 4′-*O*-sulfate derivatives using sulfotransferases [21]. Additionally, a sulfated form of taxifolin at position 7 has been isolated from a *Salix* (willow) hybrid [22]; however, no synthesis methods have been reported for this compound.

Therefore, the development of a reliable synthesis method for taxifolin sulfate conjugates is essential for the detailed identification of metabolites and exploration of their pharmacological potential. This study presents a method for the chemical synthesis of taxifolin monosulfate conjugates and identification of their chemical structures (Scheme 2).




Taxifolin 3-*O*-sulfate (**2**) ($R_1 = SO_3K$, $R_2 = R_3 = R_4 = H$)
Taxifolin 7-*O*-sulfate (**3**) ($R_2 = SO_3K$, $R_1 = R_3 = R_4 = H$ )
Taxifolin 3'-*O*-sulfate (**4**) ($R_3 = SO_3K$, $R_1 = R_2 = R_4 = H$)
Taxifolin 4'-*O*-sulfate (**5**) ($R_4 = SO_3K$, $R_1 = R_2 = R_3 = H$)

**Scheme 2:** Structure of taxifolin monosulfates.

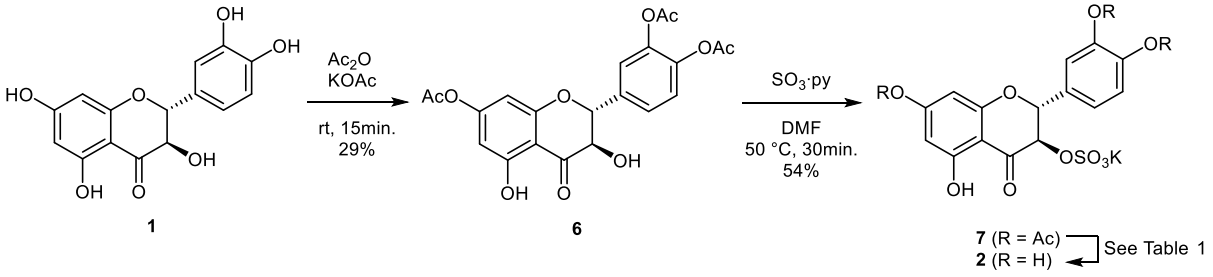
# Results and Discussion

Among the numerous metabolites of taxifolin, monosulfate derivatives are the principal metabolic forms [16,17]. Four types of taxifolin monosulfate conjugates were synthesized using taxifolin (**1**) as the starting material.

## Synthesis of taxifolin 3-*O*-sulfate

Taxifolin 3-*O*-sulfate (**2**) was synthesized using an acetyl group as the protecting group (Scheme 3). First, taxifolin (**1**) was reacted with acetic anhydride using potassium acetate as a base catalyst [23]; subsequently, taxifolin 7,3′,4′-tri-*O*-sulfate (**6**) was obtained by column chromatography and recrystallization. Next, sulfation of compound **6** using pyridine-sulfur trioxide complex afforded a mixture of taxifolin 7,3′,4′-tri-*O*-

acetyl-3-*O*-sulfate (**7**) and epitaxifolin 7,3′,4′-tri-*O*-acetyl-3-*O*-sulfate (***epi*-7**) in a ratio of 4:1. Subsequently, we optimized the reaction conditions for the deacetylation step (Table 1). Treatment with potassium carbonate and potassium hydroxide resulted in substrate decomposition (entries 1 and 2). Ammonium acetate efficiently removed the acetyl groups [24], affording the product in a 5:2 *trans*/*cis* isomer ratio (entry 3). Imidazole-mediated deacetylation also proceeded smoothly and gave the product in a 5:1 *trans*/*cis* ratio (entry 4). The use of an aqueous ammonia solution afforded a ratio comparable to that obtained with imidazole, with the additional advantage of improved ease of isolation (entry 5).




**Scheme 3:** Synthesis of taxifolin 3-*O*-sulfate (**2**).

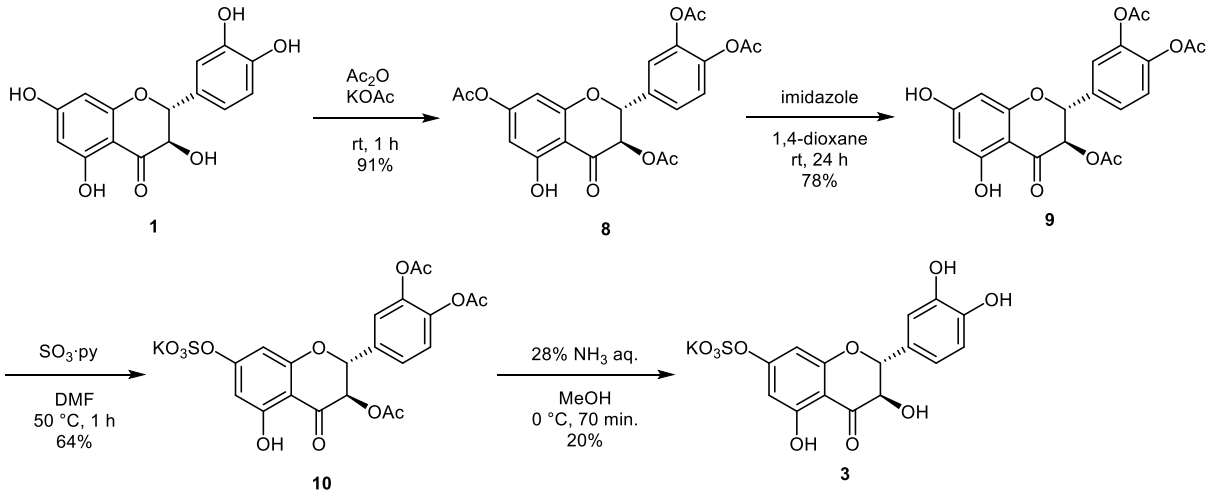
**Table 1:** Deprotection of acetyl group in compound **7**.

| Entry | Reaction conditions | Yield (%)[a] | *trans*/*cis* ratio |
|---|---|---|---|
| 1 | $K_2CO_3$, MeOH, rt, 40 min. | 0[b] | - |
| 2 | KOH, $H_2O$/MeOH, 0 °C, 15 min. | 0[b] | - |
| 3 | $NH_4OAc$, $H_2O$/MeOH, 50 °C, 10 h | 95 | 5:2 |
| 4 | Imidazole, $H_2O$/MeOH, rt, 5 h | 93 | 5:1 |
| 5 | 2 M $NH_3$ aq., acetone, 0 °C, 2 h | 97 | 5:1 |

[a] Isolated yield. [b] Decomposition was confirmed by TLC.

## Synthesis of taxifolin 7-*O*-sulfate

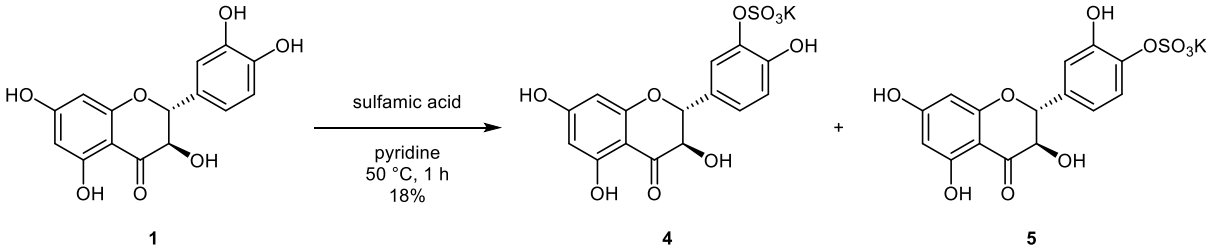
Taxifolin 7-sulfate (**3**) was difficult to obtain by direct sulfation of taxifolin (**1**), owing to the low reactivity of the hydroxy group at position 7 and the instability of the resulting sulfate ester. These characteristics are attributed to the presence of a carbonyl group at the *para*-position relative to the hydroxy group. Therefore, compound **3** was synthesized via a protecting group strategy (Scheme 4). Taxifolin was first reacted with acetic anhydride in pyridine to afford taxifolin 3,7,3′,4′-tetra-*O*-acetate (**8**) [23]. Selective deacetylation of 7-OAc was then performed using imidazole yielding taxifolin 3,3′,4′-tri-*O*-acetate (**9**) [25]. Sulfation was subsequently performed using a pyridine-sulfur trioxide complex to obtain taxifolin 3,3′,4′-tri-*O*-acetyl-7-*O*-sulfate (**10**). Deacetylation of compound **10** could be carried out using ammonia, as in the synthesis of compound **2**. However, the reaction required a long time to reach completion when a 5% aqueous ammonia solution was used. Under this condition, not only removal of the acetyl groups but also decomposition of the sulfate ester was observed, and a significant amount of taxifolin was detected as a byproduct. The use of a 28% aqueous ammonia solution enabled rapid completion of the reaction and successfully suppressed the formation of taxifolin. However, the obtained product exhibited low stability and gradually decomposed during the isolation process, making it extremely difficult to obtain pure compound **3**.




**Scheme 4:** Synthesis of taxifolin 7-*O*-sulfate (**3**).

## Synthesis of taxifolin 3′-*O*-sulfate and 4′-*O*-sulfate

Taxifolin 3′-*O*-sulfate (**4**) and taxifolin 4′-*O*-sulfate (**5**) were obtained by the following procedure (Scheme 5). Initially, taxifolin was reacted with sulfamic acid in pyridine. Subsequent reversed-phase column chromatography resulted in a mixture of compounds **4** and **5**. Further purification using HPLC afforded compounds **4** and **5** with approximately 87% purity.




**Scheme 5:** Synthesis of taxifolin 3′-*O*-sulfate (**4**) and 4′-*O*-sulfate.

## Identification

The structures of synthesized compounds **2**–**5** was confirmed by $^{1}$H NMR, $^{13}$C NMR, 2D NMR (COSY, HMQC, HMBC), LC-MS and Circular dichroism (CD) spectroscopy

(Tables 2, 3 and Schemes 5-7). Although the sulfate group is not directly observable by NMR, sulfation of the hydroxy group on taxifolin induces predictable changes in the chemical shifts of the proton and carbon atoms. These shifts enabled determination of the sulfation sites.

**Table 2:** [1]H NMR data of taxifolin **1** and taxifolin sulfates conjugates **2**-**5**.

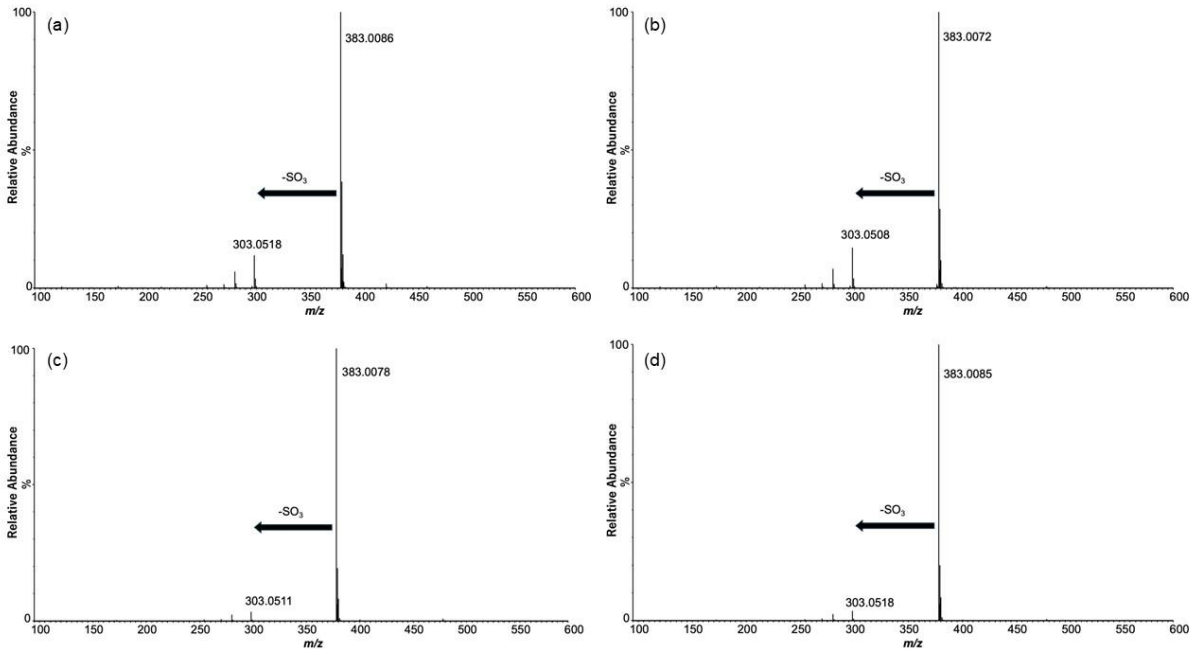
| Position | $\delta_H$ (*J* in Hz)[a] | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| 2 | 4.96 (d, 11.2) | 5.87 (d, 2.3) | 5.02 (d, 11.5) | 5.03 (d, 11.4) | 5.04 (d, 10.9) |
| 3 | 4.49 (dd, 11.2, 6.0) | 4.65 (d, 2.3) | 4.54 (dd, 11.5, 5.7) | 4.55 (dd, 11.4, 6.5) | 4.54 (dd, 10.9, 6.3) |
| 6 | 5.89 (d, 2.0) | 5.85 (d, 2.0) | 6.28 (d, 2.3) | 5.90 (d, 1.9) | 5.90 (d, 1.7) |
| 8 | 5.85 (d, 2.0) | 5.96 (d, 2.0) | 6.34 (d, 2.3) | 5.87 (d, 1.9) | 5.87 (d, 1.7) |
| 2′ | 6.86 (d, 1.2) | 6.62 (d, 1.4) | 6.88 (d, 2.3) | 7.27 (d, 2.3) | 6.97 (d, 2.3) |
| 5′ | 6.75-6.71 (m) | 6.66 (d, 8.3) | 6.76-6.72 (m) | 6.84 (d, 8.4) | 7.15 (d, 8.0) |
| 6′ | 6.75-6.71 (m) | 6.43 (dd, 8.3, 1.4) | 6.76-6.72 (m) | 7.10 (dd, 8.4, 2.3) | 6.88 (dd, 8.0, 2.3) |
| 3-OH | 5.74 (d. 6.0) | - | 5.78 (s, 5.7) | 5.77 (d, 6.5) | 5.83 (d, 6.3) |

[a] Recorded at 500 MHz in DMSO-$d_6$, 25 °C

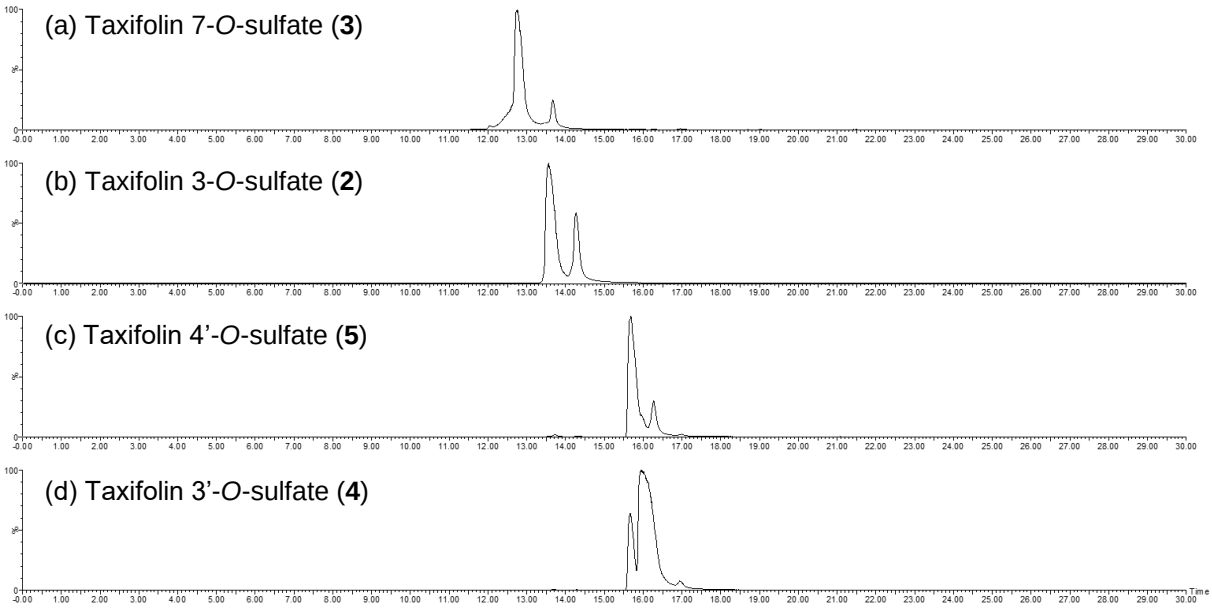
**Table 3:** $^{13}C$ NMR data of taxifolin **1** and taxifolin sulfate conjugates **2**-**5**.

| Position | $\delta_C$[a] | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** |
| 2 | 83.0 | 79.7 | 83.0 | 82.6 | 82.6 |
| 3 | 71.6 | 75.0 | 71.8 | 71.4 | 71.4 |
| 4 | 197.8 | 189.0 | 198.7 | 197.8 | 197.6 |
| 4a | 100.5 | 101.3 | 102.3 | 100.5 | 100.4 |
| 5 | 163.3 | 163.6 | 162.0 | 163.3 | 163.3 |
| 6 | 96.0 | 95.7 | 98.2 | 96.0 | 96.1 |
| 7 | 166.8 | 167.6 | 161.7 | 166.8 | 166.8 |
| 8 | 95.0 | 94.9 | 99.3 | 95.0 | 95.0 |
| 8a | 162.6 | 161.4 | 162.1 | 162.5 | 162.4 |
| 1′ | 128.0 | 126.1 | 127.9 | 128.3 | 133.8 |
| 2′ | 115.3 | 113.4 | 115.3 | 123.0 | 116.9 |
| 3′ | 144.9 | 145.3 | 144.9 | 140.5 | 148.8 |
| 4′ | 145.8 | 145.4 | 145.8 | 149.6 | 141.2 |
| 5′ | 115.1 | 115.7 | 115.1 | 116.8 | 122.6 |
| 6′ | 119.4 | 117.0 | 119.4 | 124.9 | 119.2 |

[a] Recorded in 125 MHz, DMSO-$d_6$, 25 °C.



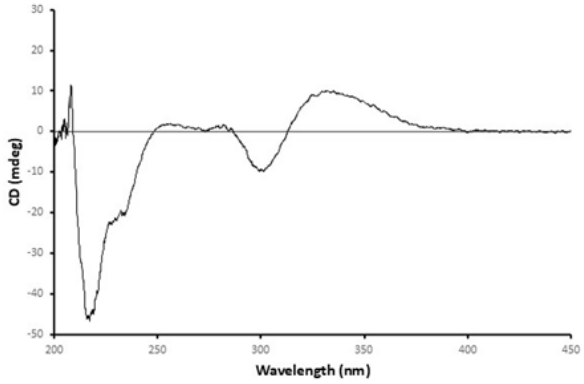

**Scheme 5:** Mass spectra of taxifolin sulfates: (a) taxifolin 3-*O*-sulfate (**2**); (b) taxifolin 7-*O*-sulfate (**3**); (c) taxifolin 3′-*O*-sulfate (**4**); (d) taxifolin 4′-*O*-sulfate (**5**).




**Scheme 6:** LC–MS mass chromatograms monitored by $(M–H)^-$ ions of taxifolin monosulfates (*m*/*z* 383.0078): (a) Taxifolin 7-*O*-sulfate (**3**); (b) Taxifolin 3-*O*-sulfate (**2**); (c) Taxifolin 4′-*O*-sulfate (**5**); (d) Taxifolin 3′-*O*-sulfate (**4**).

Compound **2** was identified as follows: $^{1}$H NMR spectrum of compound **2** exhibited signals at $\delta_H$ 6.62, $\delta_H$ 6.66, and $\delta_H$ 6.43, corresponding to the aromatic protons of the B ring at position of 2′, 5′, and 6′ respectively. Additionally, signals were observed at $\delta_H$ 5.85 and $\delta_H$ 5.95, which are attributed to the A ring protons at positions 6 and 8, respectively. Compared with the spectrum of taxifolin, the signal corresponding to 3-OH was absent in compound **2**, and the proton at position 3 changed from a double doublet (dd) to a doublet (d). This change in the splitting pattern indicated that 3-OH was functionalized. Mass spectrometry analysis of compound **2** further supported its structure, showing ion peaks at *m*/*z* 383.0086 $[M-K]^-$, and *m*/*z* 303.0518 $[M-K-SO_3]^-$ (Scheme 5a). These results suggest that compound 2 is taxifolin 3-*O*-sulfate. However, the coupling constant between the protons at positions 2 and 3 (2.3 Hz) was significantly smaller than that observed for taxifolin (11.2 Hz), suggesting a change in the stereoisomerism at these chiral centers. The inversion of the chiral center in taxifolin reportedly proceed via a ring-opening mechanism of the C-ring under both acidic and basic conditions [26]. In this process, the configuration at C-2 is inverted, resulting in a mixture of the *trans* (2*R*,3*R*) and *cis* (2*S*,3*R*) forms. Therefore, the stereochemistry of C-2 is identified from CD spectrum shown in Scheme 7. In the CD spectra of dihydroflavonols, the sign of the Cotton effect depends on the stereochemistry at C-2. Specifically, compounds with an *R* configuration at C-2, such as taxifolin, exhibit a positive Cotton effect, whereas those with an *S* configuration show a negative effect [27-29]. The measured CD spectrum of compound **2** displays a positive Cotton effect, indicating that the *R* configuration exist at C-2 of compound **2**. The observed decrease in the *J* value can be attributed to distortion of the dihedral angle caused by the steric effect of the bulky sulfate group. Based on these analytical data, compound **2** was identified as taxifolin 3-*O*-sulfate. In the LC-MS chromatogram of the product, a byproduct was confirmed (Scheme 6b). This byproduct is a

stereoisomer possessing a *cis* configuration between the position 2 and position 3, which is also observed by $^{1}$H NMR analysis.




**Scheme 7:** CD Spectrum of taxifolin 3-*O*-sulfate (**2**).

The structure of compound **3** was identified as follows: $^{1}$H NMR spectrum of compound **3** displayed aromatic proton signals attributed to the B ring at $\delta_H$ 6.88 and $\delta_H$ 6.76–6.72, corresponding to positions 2′, 5′, and 6′, respectively. Moreover, two *meta*-coupled doublet peaks at $\delta_H$ 6.28 and $\delta_H$ 6.34 were observed, corresponding to the A-ring protons at positions 6 and 8. These A-ring protons showed a downfield shift relative to taxifolin, supporting the presence of a sulfate group at position 7. The stereochemistry at the chiral centers (C-2 and C-3) was identical to that of taxifolin (**1**), as indicated by the coupling constants of the protons at positions 2 and 3. Mass spectrometry of compound **3** showed ion peaks at *m*/*z* 383.0072 $[M\text{-}K]^-$ and *m*/*z* 303.0508 $[M\text{-}K\text{-}SO_3]^-$, consistent with the presence of a sulfate moiety and confirming that the compound was monosulfated taxifolin (Scheme 5b). Based on these results, compound **3** was identified as taxifolin 7-*O*-sulfate. Both NMR and mass spectrometry data were consistent with previously reported characteristics of taxifolin 7-*O*-sulfate (**3**) [22]. LC–MS analysis revealed a byproduct with a longer retention time than compound **3** (Scheme 5a). This compound was identified as a stereoisomer (***epi*-3**), and its

presence was further confirmed by $^1$H NMR, which exhibited a smaller coupling constant between positions 2 and 3.

The structure of compounds **4** and **5** were elucidated based on the NMR and mass spectrometry data. For compound **4**, the $^1$H NMR spectrum showed aromatic B ring signals at $\delta_H$ 7.27, $\delta_H$ 6.84, and $\delta_H$ 7.10, corresponding to the 2′, 5′, and 6′ positions, respectively. Compound **5** exhibited aromatic proton signals at $\delta_H$ 6.97, $\delta_H$ 7.15, and $\delta_H$ 6.88, assigned to the B ring. Sulfation at the 3′- or 4′-hydroxy group induced characteristic downfield shift in adjacent protons: for compound **4**, the 2′ and 6′ protons shifted to downfield, whereas for compound **5**, a downfield shit was observed at the 5′ position, indicating sulfate substitution at the 3′ and 4′ positions, respectively. The A ring proton signals were observed at $\delta_H$ 5.90 and $\delta_H$ 5.87 in compound **4**, and at $\delta_H$ 5.90 and $\delta_H$ 5.87 in compound **5**. Based on the coupling constats between the protons at positions 2 and 3 (11.4 Hz for compound 4, and 10.9 Hz for compound 5), the stereochemistry was determined to be identical to that of taxifolin (**1**) The mass spectrometry results for compounds **4** and **5** further supported their structures. Peaks were observed at *m*/*z* 383.0078 $[M-K]^-$ and 303.0511 $[M-K-SO_3]^-$ for compound **4**, and at *m*/*z* 383.0085 $[M-K]^-$ and 303.0518 $[M-K-SO_3]^-$ for compound **5**, confirming both as monosulfated taxifolin (Schemes 5c and 5d). Based on these results, compound **4** was identified as taxifolin 3′-*O*-sulfate and compound **5** as taxifolin 4′-*O*-sulfate. The NMR and MS spectra were consistent with previously reported data for compounds **4** and **5** [21]. In the LC-MS chromatograms of these products, in addition to the regioisomer peak, another peak was observed in each case (Schemes 5c and 5d). This additional peak is presumed to correspond to the epimers (***epi*-4** and ***epi*-5**).

Cai et al. confirmed the presence of nine taxifolin monosulfate conjugates or their isomers in rat blood, faeces, urine, and liver as taxifolin metabolites [18]. However, their detailed chamical structure remain uncharacterized. In this study, compounds

with a *cis* configuration formed through epimerization were observed, and they appeared as distinct peaks in the LC-MS chromatogram. Therefore, among the nine monosulfate conjugates reported by Cai et al., four were considered to correspond to the taxifolin monosulfate conjugates (**2**–**5**), and the other four possibly represent their stereoisomeric forms.

# Conclusion

In this study, we synthesized, isolated, and structurally characterized four taxifolin sulfate conjugates, 3-, 7-, 3′-, and 4′-*O*-sulfates. Unlike simple excretory metabolites, taxifolin sulfate conjugates possess enhanced pharmacological activity, making them promising candidates for application in pharmaceuticals and dietary supplements. The four compounds were synthesized via simple process, highlighting the potential of this chemical approach as a practical method for producing biologically active compounds. Among the monosulfate conjugates, taxifolin 7-*O*-sulfate has been reported to exhibit notable antioxidant activity [22]. However, the biological activities of other three sulfate conjugates remain unexplored. Therefore, future studies must focus on elucidating the pharmacological significance of these newly synthesized and identified taxifolin sulfate conjugates.

# Experimental

### General Information

*Larix kaempferi* extract powder, was purchased from DHQ Co., Ltd. (Saitama, Japan). Normal-phase column chromatography was performed using Silica Gel 60N (spherical, neutral) (63-210 μm, Kanto Chemical Co., Ltd. (Tokyo, Japan)). Reversed-phase

column chromatography was performed using Universal Premium Column ODS-SM (30 μm, 3.0 × 20.0 cm, Yamazen Co., Ltd. (Osaka, Japan)) and Universal Premium Column ODS-SM (30 μm, 2.3 × 12.3 cm, Yamazen Co., Ltd. (Osaka, Japan)) on a BÜCHI Pump Module C-605 (BÜCHI (Flavil, Switzerland)). The strong acid cation exchange resin was prepared by passing 2 M hydrochloric acid through the Dowex 50W×8 200-400 mesh (H) cation exchange resin (H-form) (Fujifilm Wako Pure Chemical Corporation (Osaka, Japan)) for 5 bed volume, then washing with pure water until the pH of the eluate reached neutral. The cation exchange resin (K-form) was prepared by passing 0.5 M potassium hydroxide solution through the strong acid cation exchange resin (H-form) for 5 bed volume, followed by washing with pure water until the pH of the eluate became neutral. Solid-phase extraction was performed using a Sep-Pak Vac $C_{18}$ cartridge (20 cc) (Waters Corporation (Milford, Massachusetts, USA)). Pre-coated thin-layer chromatography (TLC) was performed using Silica gel 60 $F_{254}$ (Merck, Darmstadt, Germany) and Silica gel 60 RP-18 $F_{254}$s (Merck). Spots on the TLC plate were detected by UV irradiation (254 and 365 nm). Other analytical grade chemicals and chromatography solvents (LC-MS grade) were purchased from Fujifilm Wako Pure Chemical Corporation.

**Instrumental Information**

NMR spectra were measured using a JNM-ECZ500R/S1 (Japan Electronic Co., Ltd., Tokyo, Japan) at 500 MHz ($^{1}$H) and 125 MHz ($^{13}$C). Deuterated dimethyl sulfoxide (DMSO-$d_6$) was obtained from Euriso-Top (Saint-Aubin, France). During analysis, DMSO-$d_6$ ($^{1}$H NMR: 2.49 ppm, $^{13}$C NMR: 39.5 ppm) was used as the internal standard. LC-QTOF-MS analysis was performed using a Xevo G2-XS QTOF mass spectrometer (Waters Corporation (Milford, Massachusetts, USA)) equipped with an electrospray ionization (ESI) interface in negative ion mode. The sample temperature was maintained at 4 °C using the ACQUITY UPLC H-CLASS Plus Sample Manager FTN-

H (Waters Corporation, Milford, Massachusetts, USA). Additionally, LC-QTOF-MS analysis was performed after passing through a 0.20-μm PTFE membrane. ESI parameters were as follows: data acquisition mode, MSE; collision energy, 20-50 eV; Acquisition mass range, 50–1500 Da; cone voltage, 35 eV; capillary voltage, 2.5 kV; source temperature, 150 °C; desolvation temperature, 400 °C; cone gas flow, 100.0 L/h; desolvation gas flow, 1000.0 L/h. A TOSOH TSKgel ODS-100V column (2.0 × 150 mm, 3 μm) was used at a column temperature maintained at 30 °C. The mobile phase was a binary eluent of (A) water (containing 0.1% formic acid) and (B) acetonitrile under the following gradient conditions: A linear gradient from 5% to 100% over 0-60 minutes. Flow rate: 0.2 mL/min. HPLC analysis and fractionation were performed using a Shimadzu SPD-M20A photodiode array UV-VIS detector (Shimadzu Corporation, Kyoto, Japan). An ODS column COSMOSIL $5C_{18}$-AR-II (10 I.D. × 250 mm, 5 μm, 40 °C) was used to separate the components of the extract using a 5 mM potassium acetate aqueous solution/acetonitrile = 75:25 (v/v) mobile phase. Flow rate: 5.0 mL/min. Wavelength: 290 nm. Additionally, HPLC analysis and fractionation were performed after passing through a 0.45-μm PTFE membrane. CD spectra were measured using a JASCO J-1500 (JASCO Corporation, Tokyo, Japan) at 600-200 nm and 20 °C. A 0.05 mg/mL methanol solution of the sample was prepared and analyzed.

**Procedures**

**Isolation of Taxifolin (1)**

*Larix kaempferi* extract powder (688 mg) was purified by reversed-phase column chromatography (ODS, 3.0 × 20.0 cm, 50% methanol (v/v)). Purity of each fraction were monitored by TLC (ODS, eluent: 60% methanol (v/v)), and the fractions which show a single spot with an Rf value of 0.57 was collected. The collected fractions were concentrated under reduced pressure, and the remaining water was removed by freeze-drying. The resulting residue was further dried under vacuum for 24 h to afford

taxifolin (**1**) as a pale-yellow solid (486 mg, 1.60 mmol). $^{1}H$ and $^{13}C$ NMR, see Tables 2 and 3; HRMS (ESI) *m*/*z*: $[M-H]^-$ calcd for $C_{15}H_{11}O_7^-$ 303.0510; found 303.0512; CD (MeOH) $\lambda_{ext}$ ($\Delta\varepsilon$) 328 (+14.8), 297 (-45.7), 256 (+9.5) nm.

**Synthesis of taxifolin 3-*O*-sulfate (2) (Scheme 3)**

Synthesis of taxifolin 7,3′,4′-tri-*O*-acetate (**6**) [23]. Under a nitrogen atmosphere, potassium acetate (25.0 mg, 0.26 mmol) was added to a suspension of compound **1** (387.4 mg, 1.27 mmol) in acetic anhydride (12.7 mL). The mixture was stirred at room temperature for 15 minutes, then quenched by the addition of water (130 mL) under an ice-water bath. The resulting mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic layer was washed with water (5 × 50 mL), saturated sodium bicarbonate solution (3 × 50 mL), and brine (50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1 (v/v)). Fractions were monitored by TLC ($SiO_2$, hexane/ethyl acetate = 1:2 (v/v)), and the fraction showing a single spot (Rf 0.71) was collected. After concentration under reduced pressure, the product was further purified by recrystallization (hexane/ethyl acetate) to afford taxifolin 7,3′,4′-tri-*O*-acetate (**6**) (158.8 mg, 0.37 mmol, 29% yield). White solid; mp 147.8-148.5 °C; $^{1}H$ NMR (500 MHz, DMSO-$d_6$) δ (ppm): 11.60 (s, 1H), 7.48 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.46 (d, *J* = 1.7 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 6.39 (d, *J* = 2.3 Hz, 1H), 6.36 (d, *J* = 2.3 Hz, 1H), 6.09 (d, *J* = 6.3 Hz, 1H), 5.37 (d, *J* = 12.0 Hz, 1H), 4.79 (dd, *J* = 12.0, 6.3 Hz, 1H), 2.287 (s, 3H, OAc), 2.285 (s, 3H, OAc), 2.25 (s, 3H, OAc); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ (ppm): 199.1, 168.22 (OAc), 168.19 (OAc×2), 161.9, 161.5, 158.0, 142.3, 141.8, 135.7, 126.5,

123.5, 123.2, 104.8, 103.2, 101.7, 81.9, 71.6, 20.9 (OAc), 20.4 (OAc×2); HRMS (ESI) *m*/*z*: $[M-H]^-$ calcd for $C_{21}H_{17}O_{10}^-$ 429.0827; found 429.0815.

Synthesis of taxifolin 7,3′,4′-tri-*O*-acetyl-3-*O*-sulfate (**7**). Under a nitrogen atmosphere, pyridine–sulfur trioxide complex (224.9 mg, 1.4 mmol) was added to a solution of compound **6** (121.6 mg, 0.28 mmol) in anhydrous DMF (1.4 mL). The reaction mixture was stirred at 50 °C for 30 minutes, then cooled to room temperature. The reaction was quenched by the addition of 1 M potassium acetate solution (5.6 mL) under an ice-water bath, followed by the addition of water (21 mL). The resulting solution was loaded onto a Sep-Pak $C_{18}$ cartridge (20 cc), washed with 1 M potassium acetate solution (40 mL) and water (40 mL), and then eluted with methanol (40 mL). Methanol fraction was concentrated under reduced pressure and the residual aqueous solution was freeze-dried. The residue was purified by reversed-phase column chromatography (ODS, 3.0 × 20.0 cm, 30% methanol (v/v)). Fractions were monitored by TLC (ODS, 50% methanol (v/v)), and the fraction showing a single spot (Rf = 0.37) was collected. After concentration and freeze-drying, a mixture of taxifolin 7,3′,4′-tri-*O*-acetyl-3-*O*-sulfate (**7**) and epitaxifolin 7,3′,4′-tri-*O*-acetyl-3-*O*-sulfate (***epi*-7**) was obtained as a colorless solid (83.48 mg, 0.15 mmol, **7**:***epi*-7** = 4:1) in 54% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) δ (ppm): 11.25 (s, 1H for ***epi*-7**), 11.20 (s, 1H for **7**), 7.41 (dd, *J* = 8.4, 1.9 Hz, 1H for ***epi*-7**), 7.36 (d, *J* = 1.9 Hz, 1H for ***epi*-7**), 7.28-7.22 (m, 3H for **7**), 6.46 (d, *J* = 2.3 Hz, 1H for **7**), 6.42 (d, *J* = 1.9 Hz, 1H for ***epi*-7**), 6.37 (d, *J* = 2.3 Hz, 1H for **7**), 6.36 (d, *J* = 1.9 Hz, 1H for ***epi*-7**), 5.99 (d, *J* = 5.3 Hz, 1H for **7**), 5.88 (d, *J* = 3.1 Hz, 1H for ***epi*-7**), 4.99 (br s, 1H for ***epi*-7**), 4.95 (d, *J* = 5.3 Hz, 1H for **7**), 2.28 (s, 3H, OAc for ***epi*-7**), 2.27 (s, 3H, OAc for ***epi*-7**), 2.26 (s, 3H, OAc for **7**), 2.25 (s, 6H, OAc for **7**); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ (ppm): 191.2, 168.1 (OAc), 168.06 (OAc), 168.02 (OAc), 161.8, 160.4, 158.3, 142.0, 141.9, 133.8, 124.9, 123.9, 122.1, 105.3, 103.0, 101.6, 79.7, 74.7,

20.9 (OAc), 20.31 (OAc), 20.29 (OAc); HRMS (ESI) *m*/*z*: $[M-K]^-$ calcd for $C_{21}H_{17}O_{13}S^-$ 509.0395; found 509.0378.

Synthesis of taxifolin 3-*O*-sulfate (**2**). Taxifolin 7,3′,4′-tri-*O*-acetyl-3-*O*-sulfate (**7**) (19.7 mg, 0.036 mmol, including 20% of ***epi*-7**) was dissolved in acetone (0.36 mL), and 2 M aqueous ammonia solution (0.18 mL, 0.324 mmol) was added under an ice-water bath. The reaction mixture was stirred at 0 °C for 2 hours and then concentrated under reduced pressure. The residue was purified by reversed-phase column chromatography (ODS, 3.0 × 20.0 cm, 20% methanol (v/v)). Fractions were monitored by TLC (ODS, 40% methanol), and the fraction displaying a single spot (Rf 0.67) was collected. The collected fraction was concentrated under reduced pressure, and the remaining aqueous solution was freeze-dried. The obtained crude product was dissolved in methanol (4 mL), passed sequentially through a strong acidic cation exchange resin (H-form) and then through a cation exchange resin (K-form). The eluate was concentrated under reduced pressure to afford a mixture of taxifolin 3-*O*-sulfate (**2**) and epitaxifolin 3-*O*-sulfate (***epi*-2**) as a yellow solid (6.8 mg, 0.016 mmol, **2**:***epi*-2** = 5:1) in 44% yield. $^1$H NMR (500 MHz, DMSO-$d_6$) δ (ppm): 11.57 (s, 1H for ***epi*-2**), 11.50 (s, 1H for **2**), 10.90 (br s, 1H for **2**), 9.10 (s, 1H for **2**), 8.98 (s, 1H for **2**), 8.92 (s, 1H for ***epi*-2**), 8.91 (s, 1H for ***epi*-2**), 6.81 (d, *J* = 2.0 Hz, 1H for ***epi*-2**), 6.66 (d, *J* = 8.3 Hz, 1H for **2**), 6.64 (s, 1H for ***epi*-2**), 6.62 (d, *J* = 1.4 Hz, 1H for **2**), 6.43 (dd, *J* = 8.3, 1.4 Hz, 1H for **2**), 5.96 (d, *J* = 2.0 Hz, 1H for **2**), 5.87 (d, *J* = 2.3 Hz, 1H for **2**), 5.85 (d, *J* = 2.0 Hz, 1H for **2**), 5.84 (d, *J* = 2.0 Hz, 1H for ***epi*-2**), 5.62 (d, *J* = 4.3 Hz, 1H for ***epi*-2**), 5.05 (br s, 1H for ***epi*-2**), 4.65 (d, *J* = 2.3 Hz, 1H for **2**); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ (ppm): 189.0, 167.6, 163.6, 161.4, 145.4, 145.3, 126.1, 117.0, 115.7, 113.4, 101.3, 95.7, 94.9, 79.7, 75.0; HRMS (ESI) *m*/*z*: $[M-K]^-$ calcd for $C_{15}H_{11}O_{10}S^-$ 383.0078; found 383.0086; CD (MeOH) $\lambda_{ext}$ ($\Delta\varepsilon$) 331 (+9.5), 300 (-9.7), 217 (-46.7) nm.

**Synthesis of Taxifolin 7-*O*-Sulfate (3) (Scheme 4)**

Synthesis of taxifolin 3,7,3′,4′-tetra-*O*-acetate (**8**) [23]. Under a nitrogen atmosphere, pyridine (9.5 μL) was added to a suspension of compound **1** (288.4 mg, 0.95 mmol) in acetic anhydride (0.95 mL). The reaction mixture was stirred at room temperature for 1 hour. The reaction was then quenched by the addition of water (10 mL) under an ice-water bath, followed by the addition of ethyl acetate (10 mL) with vigorous stirring for 30 minutes. The organic layer was separated, and the aqueous layer was further extracted with ethyl acetate (2 × 20 mL). The combined organic layer was washed with water (3 × 30 mL), saturated sodium bicarbonate solution (30 mL), and brine (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:2 (v/v)). Fractions were monitored by TLC ($SiO_2$, hexane/ethyl acetate = 1:1 (v/v)), and the fractions exhibiting a single spot (Rf 0.57) was collected. After concentration, taxifolin 3,7,3′,4′-tetra-*O*-acetate (**8**) was obtained in 91% yield (407.4 mg, 0.86 mmol). White solid; mp 151.2-152.0 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ (ppm): 11.20 (s, 1H), 7.51 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.47 (d, *J* = 1.9 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 6.44 (d, *J* = 1.9 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 6.09 (d, *J* = 12.2 Hz, 1H), 5.80 (d, *J* = 12.2 Hz, 1H), 2.29 (s, 3H, OAc), 2.28 (s, 3H, OAc), 2.25 (s, 3H, OAc), 1.98 (s, 3H, OAc); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ (ppm): 192.1, 168.6 (OAc), 168.2 (OAc×2), 168.1 (OAc), 161.7, 161.4, 158.3, 142.7, 141.8, 134.0, 126.1, 123.9, 123.3, 105.1, 103.7, 102.1, 79.1, 72.2, 20.9 (OAc), 20.33 (OAc), 20.29 (OAc), 20.0 (OAc); HRMS (ESI) *m*/*z*: $[M-H]^-$ calcd for $C_{23}H_{19}O_{11}^-$ 471.0933; found 471.0925.

Synthesis of taxifolin 3,3′,4′-tri-*O*-acetate (**9**) [25]. Under a nitrogen atmosphere, compound **8** (407.4 mg, 0.86 mmol) was dissolved in 1,4-dioxane (8.6 mL), and

imidazole (117.1 mg, 1.72 mmol) was added. The reaction mixture was stirred at room temperature for 24 hours. The reaction was quenched by the addition of water (30 mL) under an ice-water bath. The mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic layer was washed with water (3 × 50 mL) and once with brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 3:2 (v/v)). Fractions were monitored by TLC ($SiO_2$, hexane/ethyl acetate = 1:1 (v/v)), and the fraction displaying a single spot (Rf 0.43) was collected. After concentration, taxifolin 3,3′,4′-tri-*O*-acetate (**9**) was obtained as a colorless solid (293.2 mg, 0.68 mmol) in 78% yield. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ (ppm): 11.30 (s, 1H), 11.00 (br s, 1H), 7.49 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 5.97 (d, *J* = 1.9 Hz, 1H), 5.96 (d, *J* = 1.9 Hz, 1H), 5.94 (d, *J* = 11.8 Hz, 1H), 5.65 (d, *J* = 11.8 Hz, 1H), 2.284 (s, 3H, OAc), 2.281 (s, 3H, OAc), 1.96 (s, 3H, OAc); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ (ppm): 190.9, 168.7 (OAc), 168.2 (OAc), 168.1 (OAc), 167.3, 163.2, 162.1, 142.6, 141.8, 134.3, 126.0, 123.8, 123.2, 100.6, 96.6, 95.5, 79.0, 71.9, 20.33 (OAc), 20.30 (OAc), 20.0 (OAc); HRMS (ESI) *m*/*z*: $[M-H]^-$ calcd for $C_{21}H_{17}O_{10}^-$ 429.0827; found 429.0829.

Synthesis of taxifolin 3,3′,4′-tri-*O*-acetyl-7-*O*-sulfate (**10**). Under a nitrogen atmosphere, pyridine–sulfur trioxide complex (78.8 mg, 0.50 mmol) was added to a solution of compound **9** (42.6 mg, 0.099 mmol) in pyridine (1 mL). The reaction mixture was stirred at 50 °C for 1 hour and then cooled to room temperature. The reaction was quenched by adding 1 M potassium acetate solution (20 mL) under an ice-water bath. The resulting solution was loaded onto a Sep-Pak $C_{18}$ cartridge (20 cc), eluted with 0.1 M potassium acetate solution (20 mL), and washed with water (40 mL). Elution was then performed with 50% methanol/water (40 mL), followed by methanol (40 mL). Each

fraction was monitored by TLC (ODS, methanol/water = 50:50, v/v), and the 50% methanol/water fraction was collected. The collected eluate was concentrated under reduced pressure, and the remaining water was removed by freeze-drying to afford taxifolin 3,3′,4′-tri-*O*-acetyl-7-*O*-sulfate (**10**) as a colorless solid (34.9 mg, 0.064 mmol) in 64% yield. $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ (ppm): 11.10 (s, 1H), 7.50 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 6.43 (d, *J* = 1.9 Hz, 1H), 6.39 (d, *J* = 1.9 Hz, 1H), 5.98 (d, *J* = 11.8 Hz, 1H), 5.71 (d, *J* = 11.8 Hz, 1H), 2.28 (s, 6H, OAc), 1.97 (s, 3H, OAc); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ (ppm): 191.6, 168.7 (OAc), 168.2 (OAc), 168.1 (OAc), 162.5, 162.1, 161.3, 142.6, 141.8, 134.3, 125.9, 123.8, 123.2, 102.5, 100.0, 98.6, 79.0, 72.2, 20.4 (OAc), 20.3 (OAc), 20.0 (OAc); HRMS (ESI) *m*/*z*: $[M-K]^-$ calcd for $C_{21}H_{17}O_{13}S^-$ 509.0395; found 509.0394.

Synthesis of taxifolin 7-*O*-sulfate (**3**). Compound **10** (54.0 mg, 0.098 mmol) was dissolved in methanol (0.79 mL), and 28% aqueous ammonia (0.79 mL) was added under an ice-water bath. The reaction mixture was stirred at 0 °C for 70 minutes, then 0.1 M potassium acetate solution (20 mL) was added. The resulting solution was loaded onto a Sep-Pak $C_{18}$ cartridge (20 cc) and eluted with water (20 mL) and methanol (20 mL). The aqueous fraction was freeze-dried, and the resulting residue was purified by reversed-phase column chromatography (ODS, 2.3 × 12.3 cm, 10% methanol/water (v/v)). TLC analysis (ODS, 30% methanol (v/v)) of each fraction was performed, and the fraction showing a single spot (Rf 0.85) was collected. The collected fraction was concentrated under reduced pressure and freeze-dried to yield a crude product (10.38 mg, 0.025 mmol). This residue was dissolved in methanol (4 mL), rapidly passed through a strong acidic cation exchange resin (H-form), followed by a strong acidic cation exchange resin (K-form). The eluent was then adsorbed onto a Sep-Pak $C_{18}$ cartridge (20 cc), washed with water (40 mL), and eluted with 30% methanol (40 mL) and methanol (40 mL). TLC analysis (ODS, 30% methanol (v/v))

was performed, and the 30% methanol/water fraction (Rf = 0.78) was collected, concentrated under reduced pressure, and freeze-dried to afford taxifolin 7-*O*-sulfate (**3**) as a yellow solid (8.34 mg, 0.020 mmol) in 20% yield. $^{1}$H and $^{13}$C NMR, see Tables 2 and 3; HRMS (ESI) *m*/*z*: $[M-K]^-$ calcd for $C_{15}H_{11}O_{10}S^-$ 383.0078; found 383.0072.

**Synthesis and Separation of Taxifolin 3′-*O*-Sulfate (4) and 4′-*O*-sulfate (5) (Scheme 5)**

Synthesis of a mixture of taxifolin 3′-*O*-sulfate and 4′-*O*-sulfate. Under a nitrogen atmosphere, sulfamic acid (67.5 mg, 0.70 mmol) was added to a suspension of compound **1** (52.9 mg, 0.17 mmol) in pyridine (0.87 mL). The mixture was stirred at 50 °C for 1 hour, then cooled to room temperature. The reaction was quenched by the addition of 1 M potassium acetate solution (3.4 mL) under an ice-water bath. Water (13 mL) was then added to the mixture, and the resulting solution was adsorbed onto a Sep-Pak $C_{18}$ cartridge (20 cc), washed with water (40 mL), and eluted with methanol (40 mL). The water fraction was re-adsorbed onto a fresh Sep-Pak $C_{18}$ cartridge, washed with water (6 mL), and again eluted with methanol (20 mL). The combined methanol fractions were concentrated under reduced pressure, and the residual water was removed by freeze-drying. The resulting residue was purified by reversed-phase column chromatography (ODS, 3.0 × 20.0 cm, 30% methanol (v/v)). TLC analysis (ODS, 40% methanol (v/v)) was performed on each fraction, and the fraction showing a single spot (Rf 0.57) was collected. The collected fraction was concentrated under reduced pressure, and the residual water was removed by freeze-drying. A mixture of taxifolin 3′-*O*-sulfate (**4**) and taxifolin 4′-*O*-sulfate (**5**) (13.2 mg, 0.031 mmol, **4**:**5** = 1:1) was obtained as a yellow solid in 18% yield.

Separation of taxifolin 3′-*O*-sulfate (**4**) and 4′-*O*-sulfate (**5**). A 10.0 mg/mL methanol solution of a mixture of compounds **4** and **5** was prepared and analyzed by HPLC (ODS

column, 5 mM potassium acetate solution/acetonitrile = 75:25 (v/v)). The HPLC analysis revealed two distinct peaks corresponding to compounds **4** and **5** with different retention times, and 6.70 mg of the mixture of compounds **4** and **5** was separated by HPLC. Each collected fraction was adsorbed onto a Sep-Pak $C_{18}$ cartridge (20 cc), washed with water (20 mL), and eluted with methanol (20 mL). The methanol eluates were concentrated under reduced pressure, and the residual water was removed by freeze-drying. Each fraction was then dissolved in methanol (4 mL), quickly passed through a strong acid cation exchange resin (H-form), followed by passage through a potassium-type cation exchange resin (K-form). The resulting solutions were concentrated under reduced pressure. From the peak with the shorter retention time, compound **5** (1.94 mg, 0.0050 mmol) was obtained as a yellow solid. From the peak with the longer retention time, compound **4** (2.54 mg, 0.0066 mmol) was obtained as a yellow solid. Taxifolin 3′-*O*-sulfate (**4**); $^{1}$H and $^{13}$C NMR, see Table 2 and 3; HRMS (ESI) *m*/*z*: $[M-K]^-$ calcd for $C_{15}H_{11}O_{10}S^-$ 383.0078; found 383.0078. Taxifolin 4′-*O*-sulfate (**5**); $^{1}$H and $^{13}$C NMR, see Table 2 and 3; HRMS (ESI) *m*/*z*: $[M-K]^-$ calcd for $C_{15}H_{11}O_{10}S^-$ 383.0078; found 383.0085.

# Supporting Information

Supporting Information File 1: $^{1}$H and $^{13}$C NMR and other characterization data for all compounds.

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