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# A new biflavonoid from *Selaginella uncinata*

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**One new biflavonoid was isolated from the 60 % ethanol extract of the dried whole herbs of *Selaginella uncinata* (Desv.) Spring. On the basis of physio-chemical properties and spectral (mainly 1 D and 2 D NMR) data, the structure of the new biflavonoid was established as 2″, 3″-dihydrorobustaflavone 4′-methyl ether (4). Along with the new biflavonoid, three known compounds robustaflavone (1), robustaflavone 4′-methyl ether (2) and tetrahydrorobustaflavone (3), were isolated. Compounds 2 and 3 were isolated from *Selaginella uncinata* (Desv.) Spring for the first time.**

***Key words*:** *Selaginella uncinata* (Desv.) Spring; biflavonoids; 2″, 3″-dihydrorobustaflavone 4′-methyl ether

## Introduction

*Selaginella uncinata* (Desv.) Spring is a Chinese herbal medicine widely distributed throughout southwest China which is been used to treat jaundice, dysentery, edema and beriberoid diseases[1]. Previous phytochemical studies of the constituents of the Selaginella genus led to the discovery of a variety of compounds, including flavonoids, ligands [2,3] and biflavonoids [4-7]. Some biflavonoids and chromone glycosides from *Selaginella uncinata* (Desv.) Spring have also been reported [8]. This study describes the isolation and structural elucidation of a new biflavonoid and three known biflavonoids from the 60% ethanol extract of dried whole herbs of *S. uncinata*. (Fig. 1)

## Experimental section

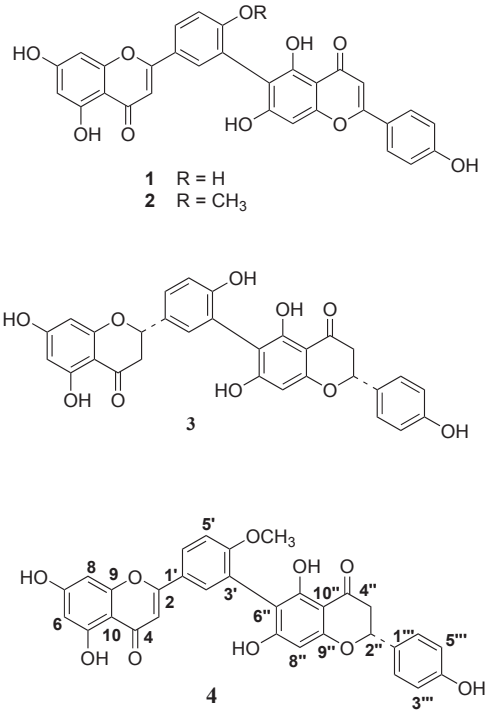


Fig. 1 The structures of compounds **1-4**

### *General experimental procedures*

Melting point (uncorrected) was determined

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with a Yanaco micro-melting point apparatus. The optical rotation was determined with a JASCOP-1020 optical rotation spectrographic apparatus. UV spectra were recorded on a Shimadzu UV2401PC spectrophotometer. IR spectra were obtained using KBr disks on a Shimadzu FTIR8900 infrared spectrometer. $^{1}H$ and $^{13}C$ NMR spectra and 2D NMR experiments were recorded on a Bruker AV-400 spectrometer at 400 and 100 MHz with tetramethylsilane as an internal standard. ESI-MS were obtained on a Bruker esquire 2000 Trap mass spectrometer. HPLC was carried out on a Shimadzu 10A/VP Series high performance liquid chromatograph. Silica-gel (Qing Dao Hai Yang Chemical Group Co., Qingdao, China) was used for column chromatography and Si gel $GF_{254}$ was used for TLC. ODS-A120-S150 was purchased from YMC Co., Ltd. Methanol for HPLC was purchased from Tianjin Kermel Chemical Agent Co., Ltd. All water used was deionized.

### *Plant material*

Herbs of *Selaginella uncinata* (Desv.) Spring were collected in Guangxi Province, P.R.China, in August 2004. The identification of the plants was confirmed by Professor Qishi Sun (Shenyang Pharmaceutical University, Shenyang). A voucher specimen (No. Y01156SU) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

### *Extraction and isolation*

The dried whole herbs (4.2 kg) of *Selaginella uncinata* (Desv.) Spring were extracted with 60 % ethanol. The extract was concentrated under vacuum to give a viscous residue (856 g) which was dissolved in water and partitioned successively with equal volumes of EtOAc and n-BuOH three times. Three fractions, EtOAc- (160 g), n-BuOH- (90.4 g) and $H_2O$-soluble (600 g) fractions, were obtained. The EtOAc-soluble fraction was subjected to column chromatography over silica-gel (200-300 mesh) and eluted with a $CH_3Cl$-MeOH gradient to give 15 fractions. Compound 1 (50 mg) and compound 2 (250 mg) were obtained from Fr. 15 (500 mg) by RP-18 HPLC. Fr. 8 (19.2 g) was subjected to Sephadex LH-20 column chromatography using $CH_3Cl$-MeOH (1:1) to yield 3 subfractions and subfraction 2 was further isolated by ODS, Rp-18 HPLC and crystallization to give compound 3 (30 mg) and compound 4 (15 mg).

## Results and discussion

Compound **4** was obtained as as an amorphous yellow powder. The Mg-HCl reaction was positive, which confirmed that **4** was a flavone. $[\alpha]_D^{29}$ +3.48° (DMSO, *c* 1). Its UV absorptions in methanol were at $\lambda_{max}$ (nm) 335 (log ε 4.28), 292 (log ε 4.37) and 269 (log ε 4.36). Its IR absorptions showed the presence of hydroxyl (3354 $cm^{-1}$), conjugated carbonyl (1652), and aromatic rings (1605, 1493 and 1439 $cm^{-1}$). The positive and negative ESI-MS of **4** gave the quasi-molecular ion at *m/z* 555 $[M+H]^+$ and *m/z* 553 $[M-H]^-$, respectively. Thus, its molecular formula was deduced to be $C_{31}H_{22}O_{10}$ using a combination of $^{1}H$ NMR and $^{13}C$ NMR, and this was also confirmed by HR-ESI-MS (found pseudomolecular ion at *m/z* 577.1152 $[M+Na]^+$, calcd 577.1111). In the $^{1}H$ NMR spectrum（400 MHz,， DMSO-$d_6$) (Table 1), a one-proton singlet at $\delta$ 6.83 (H-3) and three double doublets at $\delta$ 5.48 (H-2″), 3.25 (H-3″α), and 2.71 (H-3″β) exhibited characteristic of a flavone and flavanone unit [9]. An ABX coupling system signals, appearing with signals at $\delta$ 8.05 (1H, *dd*, *J* = 8.8, 2.3 Hz, H-6′), 7.75 (1H, *d*, *J* = 2.3 Hz, H-2′), and 7.21 (1H, *d*, *J* = 8.8 Hz, H-5′), indicated that C-3′ was the site of linkage [10]. The $^{1}H$ NMR spectrum clearly showed that the following proton systems are implicated in the structure: two meta-coupled proton signals at H-6 and H-8 appeared at $\delta$ 6.19 and 6.49 (*J* = 2.2 Hz), and an AA′XX′ coupling system signals at $\delta$ 6.81 (2H, *d*, *J* = 8.6 Hz, H-3‴, -5‴) and 7.35 (2H, *d*, *J* = 8.6 Hz, H-2‴, -6‴). Two chelated hydroxyl groups ($\delta$ 12.94, 1H, *br s* and 12.39, 1H, *br s*) and a methoxyl group ($\delta$ 3.79, 3H, *s*) were also identified in the $^{1}H$ NMR spectrum. Furthermore, the two proton signals appearing at $\delta$ 6.83 (1H, *s*, H-3) and 6.05 (1H, *s*, H-8″) were assigned in the HMBC experiment. The $^{13}C$ NMR

Table 1 $^{1}$H NMR Data of Compounds **1–4** [a]

| Position | **4** | **3** | **2** | **1** |
|---|---|---|---|---|
| 2 | | 5.47 (1H, *dd*, *J* =13.3, 2.9 Hz ) | | |
| 3 | 6.83 (1H, *s*) | 3.32 (1H, *dd*, *J* =17.2, 12.6 Hz )<br>2.72 (1H, *dd*, *J* =17.2, 2.9 Hz ) | 6.86 (1H, *s*) | 6.78 (1H, *s*) |
| 6 | 6.19 (1H, *d*, *J* =2.2 Hz ) | 5.88 (1H, *d*, *J* =2.1 Hz ) | 6.20 (1H, *d*, J =2.1 Hz) | 6.19 (1H, *d*, *J* =2.1 Hz ) |
| 8 | 6.49 (1H, *d*, *J* =2.2 Hz ) | 5.89 (1H, *d*, *J* =2.1 Hz ) | 6.50 (1H, *d*, *J* =2.1 Hz) | 6.48 (1H, *d*, *J* =2.1 Hz ) |
| 2′ | 7.75 (1H, *d*, *J* =2.3 Hz ) | 7.13 (1H, *d*, *J* =2.3 Hz ) | 7.84 (1H, *d*, *J* =2.4 Hz) | 7.79 (1H, *d*, *J* =2.4 Hz ) |
| 5′ | 7.21 (1H, *d*, *J* =8.8 Hz ) | 6.88 (1H, *d*, *J* =8.4 Hz ) | 7.24 (1H, *d*, *J* =8.9 Hz) | 7.04 (1H, *d*, *J* =8.7 Hz ) |
| 6′ | 8.05 (1H, *dd*, *J* =8.8, 2.3 Hz ) | 7.29 (1H, *dd*, *J* =8.4, 2.3 Hz ) | 8.08 (1H, *dd*, *J*=8.9, 2.4 Hz) | 7.91 (1H, *dd*, *J* =8.7, 2.4 Hz ) |
| 2″ | 5.48 (1H, *dd*, *J* =12.5, 2.8 Hz ) | 5.43 (1H, *dd*, *J* =13.3, 2.8 Hz ) | | |
| 3″ | 3.25 (1H, *dd*, *J* =17.0, 12.5 Hz )<br>2.71 (1H, *dd*, *J* =17.0, 2.8 Hz ) | 3.26 (1H, *dd*, *J* =17.1, 12.2 Hz )<br>2.66 (1H, *dd*, *J* =17.1, 2.8 Hz ) | 6.81 (1H, *s*) | 6.81 (1H, *s*) |
| 8″ | 6.05 (1H, *br s*) | 6.04 (1H, *s*) | 6.64 (1H, *s*) | 6.63 (1H, *s*) |
| 2‴ / 6‴ | 7.35 (2H, *d*, *J* =8.6 Hz ) | 7.35 (2H, *d*, *J* =8.6 Hz ) | 7.95 (2H, *d*, *J* =8.8 Hz) | 7.96 (2H, *d*, *J* =8.8 Hz ) |
| 3‴ / 5‴ | 6.81 (2H, *d*, *J* =8.6 Hz ) | 6.81 (2H, *d*, *J* =8.6 Hz ) | 6.95 (2H, *d*, *J* =8.8 Hz) | 6.95 (2H, *d*, *J* =8.8 Hz ) |
| OH-5 | 12.94 (1H, *br s*) | 12.16 (1H, *br s*) | 12.90 (1H, *br s*) | 12.99 (1H, *br s*) |
| OH-5″ | 12.39 (1H, *br s*) | 12.38 (1H, *br s*) | 13.20 (1H, *br s*) | 13.23 (1H, *br s*) |
| OH-4‴ | | 9.62 (1H, *br s*) | | |
| OMe-4′ | 3.79 (3H, *s*) | | 3.80 (3H, *s*) | |

[a] Measured in 400 MHz, DMSO-$d_6$; multiplicity and coupling constant (*J* in Hz ) assigned in parentheses; *br s*, broad singlet; *d*, doublet; *dd*, double doublet; *s*, singlet.

spectrum (100 MHz, DMSO-$d_6$) (Table 2) showed signals for all 31 carbons of the molecule, including two carbonyl groups ($\delta$ 196.3 and 181.8) and one methoxyl group ($\delta$ 55.8). In the HMBC spectrum (Fig. 2), the proton signal $\delta$ 12.39 (H-5″-OH) proton signal was correlated with the carbon signals at $\delta$ 105.6 (C-6″) and 101.4 (C-10″), $\delta$ 7.75 (H-2′) proton signal at $\delta$ 130.5 was correlated with the carbon signals at $\delta$ 160.9 (C-4′), 127.7 (C-6′) and 105.6 (C-6″), $\delta$ 6.05 (H-8″) proton signal at $\delta$ 94.7 was correlated with the carbon signals at $\delta$ 162.0. (C-7″), 160.8 (C-9″), 105.6 (C-6″) and 101.4 (C-10″), indicating that **4** was a biflavonoid with a C-3′ -C-6″ interflavonoid linkage corresponding to the robustaflavone series [11]. The HMBC experiment

Table 2 $^{13}$C-NMR Data of Compounds **1–4** [a]

| Position | 4 | 3 | 2 | 1 |
|---|---|---|---|---|
| **2** | 163.5 | 78.6 | 163.4 | 163.9 |
| 3 | 103.5 | 42.1 | 103.5 | 102.8 |
| 4 | 181.8 | 196.5 | 181.7 | 181.7 |
| 5 | 161.5 | 163.5 | 161.4 | 161.5 |
| 6 | 99.0 | 95.8 | 96.8 | 98.8 |
| 7 | 164.4 | 166.6 | 164.1 | 164.1 |
| 8 | 94.2 | 94.9 | 94.1 | 94.0 |
| 9 | 157.4 | 163.0 | 157.4 | 157.4 |
| 10 | 103.7 | 101.8 | 103.7 | 103.7 |
| 1′ | 122.9 | 128.2 | 122.4 | 120.8 |
| 2′ | 130.5 | 131.4 | 130.3 | 130.9 |
| 3′ | 122.3 | 120.0 | 122.6 | 121.0 |
| 4′ | 160.9 | 156.2 | 160.6 | 159.8 |
| 5′ | 111.7 | 115.4 | 111.7 | 116.2 |
| 6′ | 127.7 | 127.1 | 127.8 | 127.5 |
| 2″ | 78.5 | 78.4 | 163.7 | 163.6 |
| 3″ | 42.1 | 42.0 | 102.8 | 102.8 |
| 4″ | 196.3 | 196.4 | 181.8 | 181.8 |
| 5″ | 160.9 | 161.1 | 158.9 | 159.1 |
| 6″ | 105.6 | 106.6 | 108.6 | 109.0 |
| 7″ | 162.0 | 164.8 | 161.9 | 162.4 |
| 8″ | 94.7 | 94.6 | 93.4 | 93.5 |
| 9″ | 160.8 | 161.6 | 156.3 | 156.4 |
| 10″ | 101.4 | 101.5 | 103.5 | 103.5 |
| 1‴ | 129.0 | 129.0 | 121.2 | 121.3 |
| 2‴ | 128.4 | 128.4 | 128.5 | 128.5 |
| 3‴ | 115.2 | 115.2 | 115.9 | 116.0 |
| 4‴ | 157.8 | 157.7 | 161.1 | 161.2 |
| 5‴ | 115.2 | 115.2 | 115.9 | 116.0 |
| 6‴ | 128.4 | 128.4 | 128.5 | 128.5 |
| OMe-4′ | 55.8 | | 55.8 | |

[a] Measured in 100 MHz, DMSO-$d_6$

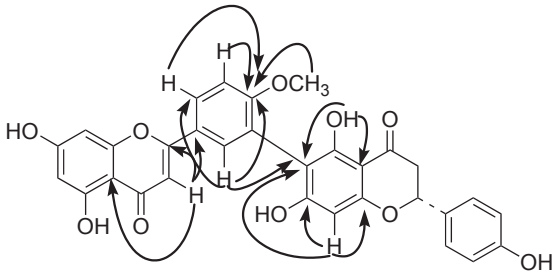


Fig. 2 The Key HMBC Correlations of Compound **4**

demonstrated that $\delta$ 6.83 (H-3) was correlated with $\delta$ 163.5 (C-2), 122.9 (C-1′), and 103.7 (C-10). H-2′ showed correlations with $\delta$ 160.9 (C-4′), 122.9 (C-1′), and 122.3 (C-3′), H-5′ with $\delta$ 160.9 (C-4′), H-6′ with $\delta$ 160.9 (C-4′) and 130.5 (C-2′), and $OCH_3$-4′ with $\delta$ 160.9 (C-4′), which confirmed the presence of a -$OCH_3$ group at C-4′. Therefore, the structure of compound 4 was clearly established as 2″, 3″-dihydrorobustaflavone 4′ -methyl ether.

Compound **3** was obtained as a yellow needles, mp 250–252 ºC. The Mg-HCl reaction was positive, which confirmed that **3** was a flavone. $[\alpha]_D^{29}$ +2.76° (DMSO, *c* 1). Its UV absorptions in methanol are at $\lambda_{max}$ (nm) 289 (log ε 4.91), 224 (log ε 5.06) and 211 (log ε 5.08). Its IR absorptions showed the presence of hydroxyl (3394 $cm^{-1}$), conjugated carbonyl (1643), and aromatic rings (1597, 1516, 1493 and 1458 $cm^{-1}$). The negative ESI-MS of **3** gave the quasi-molecular ion at *m/z* 541 $[M-H]^-$. Thus, its molecular formula was deduced to be $C_{30}H_{22}O_{10}$ using the combination of $^1$H NMR and $^{13}$C NMR. In the $^1$H NMR (400 MHz, DMSO-$d_6$) (Table 1): $\delta$ 12.38 (1H, *br s*, H-5″-OH), 12.16 (1H, *br s*, H-5-OH), 9.62 (1H, *br s*, H-4‴-OH), 7.35 (2H, *d, J* = 8.6 Hz, H-2‴, 6‴), 7.29 (1H, *dd*, *J* = 8.4, 2.3 Hz, H-6′), 7.13 (1H, *d*, *J* =2.3 Hz, H-2′), 6.88 (1H, *d*, *J* = 8.4 Hz, H-5′), 6.81 (2H, *d*, *J* = 8.6 Hz , H-3‴, 5‴), 6.04 (1H, *s*, H-8″), 5.89 (1H, *d*, *J* = 2.1 Hz, H-8), 5.88 (1H, *d*, *J* = 2.1 Hz, H-6), 5.47 (1H, *dd*, *J* =13.3, 2.9 Hz, H-2), 5.43 (1H, *dd*, *J* =13.3, 2.8 Hz, H-2″ ), 3.32 (1H, *dd, J* =17.2, 12.6 Hz, H-3α), 3.26 (1H, *dd*, *J* =17.1, 12.2 Hz, H-3″α), 2.72 (1H, *dd*, *J* =17.2, 2.9 Hz, H-3β), 2.66 (1H, *dd*, *J* =17.1, 2.8 Hz, H-3″β); The $^1$H NMR and $^{13}$C NMR spectral data of Compound **3** (Table 2) were consistent with the reported literature [12]. Therefore, the structure of compound **3** was determined to be tetrahydrorobustaflavone.

Compound **2** was obtained as a yellow powder. The Mg-HCl reaction was positive, which confirmed that **2** was a flavone. Its UV absorptions in methanol are at $\lambda_{max}$ (nm) 338 (log ε 2.27) and 269 (log ε 2.22). The negative ESI-MS of **2** gave the quasi-molecular ion at *m/z* 551 $[M-H]^-$. Thus, its molecular formula was deduced to be $C_{31}H_{20}O_{10}$ with the combination of $^1$H NMR and $^{13}$C NMR. In the $^1$H NMR (400 MHz, DMSO-$d_6$) (Table 1): $\delta$: 13.20 (1H, *br s*, H-5″-OH), 12.90 (1H, *br s*, H-5-OH), 8.08 (1H, *dd*, *J* = 8.9, 2.4 Hz, H-6′), 7.95 (2H, d, *J* = 8.8 Hz, H-2‴, 6‴), 7.84 (1H, *d*, *J* = 2.4 Hz, H-2′), 7.24 (1H, *d*, *J* = 8.9 Hz, H-5′), 6.95 (2H, d, *J* = 8.8 Hz, H-3‴, 5‴), 6.86 (1H, *s*, H-3), 6.81 (1H, *s*, H-3″), 6.64 (1H, *s*, H-8″), 6.50 (1H, *d*, *J* = 2.1 Hz, H-8), 6.20 (1H, *d*, *J* = 2.1 Hz, H-6), 3.80 (3H, *s*, H-4′-$OCH_3$). The $^1$H NMR and $^{13}$C NMR spectral data of Compound **2** (Table 2) were consistent with the reported literature[13]. Therefore, the structure of compound **2** was determined to be robustaflavone 4′-methyl ether.

Compound **1** was obtained as a yellow powder. The Mg-HCl reaction was positive, which confirmed that **1** was a flavone. Its UV absorptions in methanol are at $\lambda_{max}$ (nm) 342 (log ε 2.99) and 269 (log ε 2.92). The negative ESI-MS of **1** gave the quasi-molecular ion at *m/z* 537 $[M-H]^-$. Thus, its molecular formula was deduced to be $C_{30}H_{18}O_{10}$ with the combination of $^1$H NMR and $^{13}$C NMR. In the $^1$H NMR (400 MHz, DMSO-$d_6$) (Table 1): $\delta$ 13.23 (1H, *br s*, H-5″-OH), 12.99 (1H, *br s*, H-5-OH), 7.96 (2H, *d*, *J* = 8.8 Hz, H-2‴, 6‴), 7.91 (1H, *dd*, *J* = 8.7, 2.4 Hz, H-6′), 7.79 (1H, *d*, *J* = 2.4 Hz, H-2′), 7.04 (1H, *d*, *J* = 8.7 Hz, H-5′), 6.95 (2H, *d*, *J* = 8.8 Hz, H-3‴, 5‴), 6.81 (1H, *s*, H-3″), 6.78 (1H, *s*, H-3), 6.63 (1H, *s*, H-8″), 6.48 (1H, *d*, *J* = 2.1 Hz, H-8), 6.19 (1H, *d*, *J* = 2.1 Hz, H-6); The $^1$H NMR and $^{13}$C NMR spectral data of compound **1** (Table 2) were consistent with the reported literature [14]. Therefore, the structure of Compound **1** was determined to be robustaflavone.

Four biflavonoids, robustaflavone (**1**), robustaflavone 4′-methyl ether (**2**), tetrahydrorobustaflavone (**3**) and 2″, 3 ″-dihydrorobustaflavone 4′-methyl ether (**4**) were isolated from the EtOAc-soluble fraction of the 60 % ethanol extract of dried whole herbs of *Selaginella uncinata* (Desv.) Spring. Compound **4** was a new compound. Compounds **2** and **3** were isolated from the *Selaginella uncinata* (Desv.) Spring for the first time.

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