An improved synthesis of 6-*o*-methyl-scutellarein through selective benzylation

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An improved synthesis of 6-*O*-methyl-scutellarein is described. Benzyl bromide was selected to protect both the hydroxyl groups at C-4' and C-7 in scutellarein. The product was then methylated and deprotected to produce the target compound in high yield in four steps.

Keywords: scutellarin, scutellarein, 6-*O*-methyl-scutellarein, metabolite, selective benzylation

Ischemic cerebrovascular disease is a leading cause of disability and death worldwide.[1] Increasing evidence suggests that thrombin plays an important role in causing this disease.[2] It catalyses the proteolytic cleavage process in which the soluble plasma-protein fibrinogen forms insoluble fibrin, thus leading to clot formation.[3] Furthermore, oxidative stresses also play a critical role in ischaemic cerebrovascular disease.[4,5] The highly reactive oxygen species (ROS) including the singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical ($^\bullet OH$), nitric oxide ($NO^\bullet$) and hydrogen peroxide (H_2O_2) may increase the low-density lipoprotein (LDL) level and impair the bioactions that are mediated by the endothelial derived relaxing factor (nitric oxide, NO).[6]

Consequently, some drugs with anticoagulant activity and antioxidant capacity have been proposed to treat cerebrovascular disease. Traditional Chinese medicines (TCM) can be used to reveal lead compounds because they have been used in clinics for thousands of years. Breviscapine, which is a natural drug containing total flavonoids in *Erigeron breviscapus* (Vant.) Hand-Mazz. (Compositae), has been widely used in China to treat angina pectoris, cerebral infarction and coronary heart disease.[7] Pharmacological studies showed that scutellarin (**1**) (Fig. 1), which is the main active ingredient (>85%) in breviscapine, exhibited anticoagulant and antioxidant activities which reduced neuronal damage, and could be used to treat brain injury induced by cerebral ischemia/reperfusion.[8-10]

However, the results of pharmacokinetic studies on scutellarin (**1**) indicated that its oral bioavailability was very poor[11] in humans.[12] The main cause was that scutellarin (**1**) is easily hydrolysed into scutellarein (**2**) (Fig. 1) in the gastrointestinal tract before absorption *in vivo*, and scutellarein (**2**) was then converted into its methylated, sulfated or glucuronidated metabolites[13] in the blood. One circulating metabolite of scutellarin (**1**) *in vivo*, 6-*O*-methyl-scutellarein (**3**) (Fig. 1) might have some interesting therapeutic effects. Hence the synthesis of this metabolite (**3**) in large amount is very important for the further study of its biological activities.

Recently we reported a synthesis of 6-*O*-methyl-scutellarein (**3**) (Fig. 2),[14] in which dichlorodiphenylmethane was used first to protect the two adjacent hydroxyl groups at C-6 and C-7 positions in scutellarein (**2**). Then benzyl bromide was used to selectively and successively protect the hydroxyl group at C-4' in **4** and C-7 positions in **5**. Finally, 6-*O*-methyl-scutellarein (**3**) was obtained in high yield after methylation of the hydroxyl group at C-6 followed by removal of the two benzyl groups at C-7 and C-4' by hydrogenation. Unfortunately, using dichlorodiphenylmethane to selectively protect

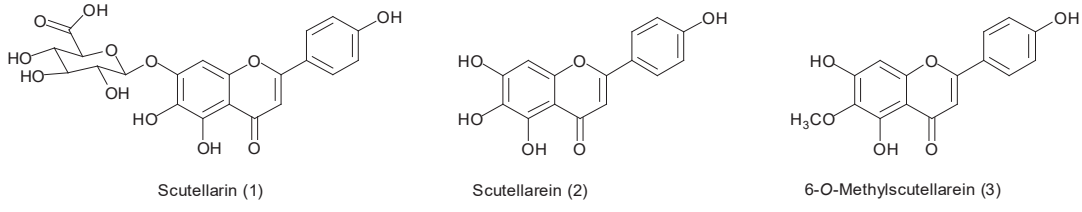

Fig. 1 The chemical structures of scutellarin (**1**), scutellarein (**2**) and 6-*O*-methyl-scutellarein (**3**).

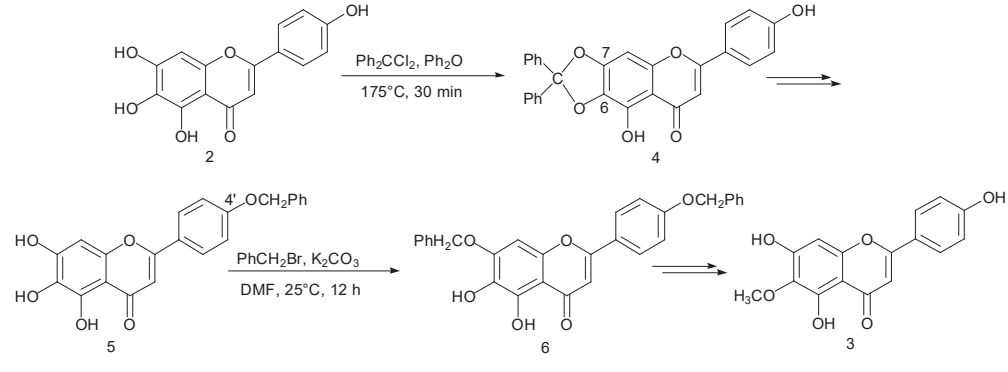

Fig. 2 The previous synthetic route of 6-*O*-methyl-scutellarein (**3**).

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the two adjacent hydroxyl groups at the C-6 and C-7 positions in scutellarein (**2**) at 175 °C in the previous synthesis precluded the preparation of 6-*O*-methyl-scutellarein (**3**) on the large-scale. Therefore, we optimised the reaction conditions and used the benzyl bromide to directly protect the two adjacent hydroxyl groups at C-4' and C-7 positions in **2**. 6-*O*-Methyl-scutellarein (**3**) was obtained after methylation and deprotection in high yield. We now describe this new synthetic route in detail.

Results and discussion

As shown in Scheme 1, scutellarein (**2**) was obtained from scutellarin (**1**) by hydrolysis with 6N HCl in 90% ethanol under reflux.[15,16] The reactivity of the four hydroxyl groups in scutellarein (**2**) were different and followed the order: 4′ > 7 > 6 >5. Direct benzylation of scutellarein (**2**) with benzyl bromide led mainly to the formation of dibenzylated product **6**. We optimised the reaction conditions as shown in Table 1.

First, the DMF was used as a solvent in this benzylation. When the reaction temperature was set at 25 ºC, the 4′,7-dibenzyl scutellarein (**6**) was obtained in 33.4% yield (Table 1, run 1) in the presence of 2.2 equiv. benzyl bromide and 2.4 equiv. K_2CO_3. However, the yield of the target compound was reduced to 28.8% and 25.9% when the reaction temperature was raised to 40 ºC and 60 ºC (Table 1, runs 2 and 3). The amount of benzyl bromide played a crucial role in this selective benzylation. We found that on decreasing (Table 1, run 4) or increasing (Table 1, run 5) the ratio of benzyl bromide, the yields of the 4′,7-dibenzyl scutellarein (**6**) were both reduced.

Although this benzylation in DMF selectively afforded the 4′,7-dibenzyl scutellarein (**6**) as the main product, the yield was not high, and a great deal of ethyl acetate and water were used to separate the main product from the DMF solution. In order to avoid this complicated process of extraction and separation, we chose acetone as a solvent for this reaction and the crude product could be separated by direct filtration. At first, we tried this selective benzylation in acetone at room temperature, but unfortunately, there was no indication of product formation even when the reaction time was prolonged to 24 h (Table 1, run 6). To make this reaction proceed easily, we increased the temperature to accelerate this benzylation. The result showed that a high yield of 53.8% was obtained within 6 h when this reaction was carried out at reflux (Table 1, run 7). When the molar ratio of benzyl bromide was decreased to 2.0 equiv. (Table 1, run 8), the yield of the 4′,7-dibenzyl scutellarein (**6**) was reduced to 31.3% because some by-products of 4′-benzyl scutellarein and 7-benzyl scutellarein were found in the reaction mixture. Furthermore, when the molar ratio of the benzyl bromide was increased (Table 1, run 9), the yield of the 4′,7-dibenzyl scutellarein (**6**) was reduced to 27.5% and the by-product of 4′,6,7-tribenzyl scutellarein was detected by TLC in the reaction mixture.

Table 1 Optimisation of reaction conditions in the synthesis of 6

Run	Reaction conditions	Yield/%
1	BnBr (2.2 equiv.), K_2CO_3 (2.4 equiv.), DMF, 25 °C , N_2, 8 h	33.4
2	BnBr (2.2 equiv.), K_2CO_3 (2.4 equiv.), DMF, 40 °C , N_2, 8 h	28.8
3	BnBr (2.2 equiv.), K_2CO_3 (2.4 equiv.), DMF, 60 °C , N_2, 8 h	25.9
4	BnBr (2.0 equiv.), K_2CO_3 (2.2 equiv.), DMF, 25 °C , N_2, 8 h	27.5
5	BnBr (2.4 equiv.), K_2CO_3 (2.6 equiv.), DMF, 25 °C , N_2, 8 h	31.3
6	BnBr (2.2 equiv.), K_2CO_3 (2.4 equiv.), acetone, r.t., N_2, 24 h	No product
7	BnBr (2.2 equiv.), K_2CO_3 (2.4 equiv.), acetone, reflux, N_2, 6 h	53.8
8	BnBr (2.0 equiv.), K_2CO_3 (2.2 equiv.), acetone, reflux, N_2, 8 h	31.3
9	BnBr (2.4 equiv.), K_2CO_3 (2.6 equiv.), acetone, reflux, N_2, 8 h	27.5

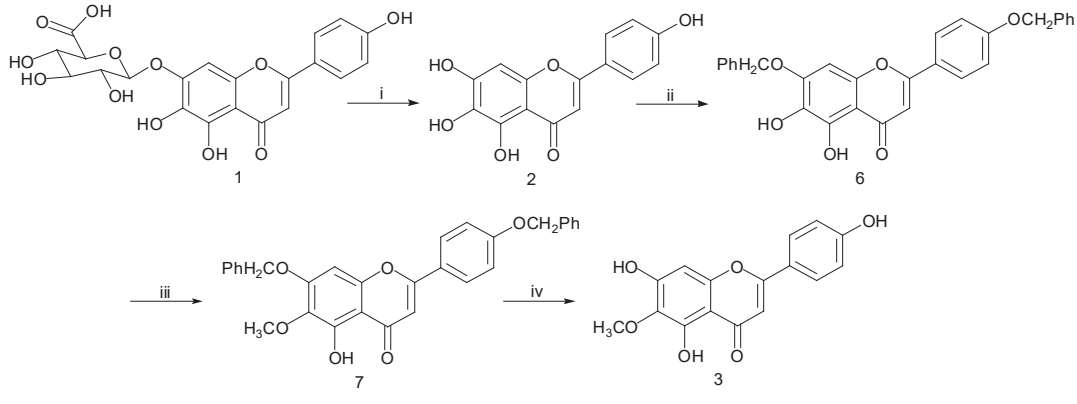
When the 4′,7-dibenzyl scutellarein (**6**) was obtained, we synthesised the 6-*O*-methyl-scutellarein (**3**) according to our previous procedure. The methylation of **6** with iodomethane (1.2 equiv.) afforded **10** in 94% yield and the only methyl group was at the C6-OH position. Removal of both the benzyl groups by hydrogenation with 10% palladium on carbon catalyst in THF/EtOH solution produced the target compound **3** in 96% yield.

Conclusion

In conclusion, we have developed an improved process for the synthesis of 6-*O*-methyl-scutellarein (**3**) in only four steps. Since the reactivity of the four hydroxyl groups was in the order: 4′ > 7 > 6 > 5, benzyl bromide was selected to protect both hydroxyl groups at C-4' and C-7. This synthetic procedure was very efficient which could be used for the selective synthesis of other *O*-methyl-flavonoid derivatives as well as the other metabolites of sulfate- and glucuronide-flavonoid.

Experimental

Reagents and solvents were used directly without further purification unless otherwise stated. Air- and moisture-sensitive solvents were transferred through a syringe or stainless steel cannula. The organic solutions were concentrated by rotary evaporation at ~20 mm Hg and below 45 °C. All the non-aqueous reactions were performed in dry and freshly distilled solvents using flame-dried glassware under a nitrogen atmosphere. Yields were calculated for chromatographically homogeneous materials. The reaction process was monitored by TLC which was conducted on 0.15–0.20 mm Yantai silica gel plates (RSGF 254) using UV light to visualise. Chromatographic separation was carried out on Qingdao silica gel (160–200 mesh) using a mixture of petroleum ether (60–90) and ethyl acetate as eluant. Melting points (m.p.) were recorded on a WRS-1B apparatus. The ^{1}H NMR and ^{13}C NMR spectra



Scheme 1 Reagents and conditions: (i) HCl, EtOH, N_2, reflux, 48h, 17%; (ii) BnBr (2.2 equiv.), K_2CO_3 (2.4 equiv.), acetone, reflux, 6h, 53.8%; (iii) CH_3I (1.2 equiv.), K_2CO_3 (1.4 equiv.), DMF, 25 °C, 6h, 94%; (iv) Pd/C (10 wt%), H_2 (1 atm), THF/EtOH, 8h, 96%.

were obtained from Bruker AV-300. The chemical shifts downfield from TMS were recorded in ppm. The *J* values were shown in Hz, abbreviations used were s (singlet), d (doublet), t (triplet), q (quartet), b (broad) and m (multiplet) respectively. ESI-MS spectra were obtained from a Synapt HDMS spectrometer.

5,6,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one **(2)**: Concentrated hydrochloric acid (120 mL) and water (10 mL) were added to a solution of **1** (10.0 g, 21.6 mmol) in ethanol (120 mL), and the reaction mixture was refluxed for 36 h under a N_2 atmosphere. The reaction mixture was cooled to room temperature, and poured into cold water. The solid which appeared was filtered, and was then purified by chromatography on the silica gel column using 50% ethyl acetate in petroleum ether afforded **2** (1.05 g, 17.0% yield) as yellow solid; m.p. 288–290 °C (lit.[17] 290–293 °C). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.57 (s, 1H, C8H), 6.74 (s, 1H, C3H), 6.91 (d, *J* = 8.8 Hz, 2H, C3',C5'H), 7.90 (d, *J* = 8.8 Hz, 2H, C2',C6'H), 8.71 (s, 1H, C6OH), 10.29 (s, 1H, C7OH), 10.44 (s, 1H, C4'OH), 12.78 (s, 1H, C5OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 98.53 (C8), 102.27 (C3), 103.22 (C10), 115.76 (C3',C5'), 121.37 (C1'), 124.91 (C6), 128.47 (C2',C6'),145.42 (C9), 152.98 (C5), 153.28 (C7), 160.99 (C4'), 163.47 (C2), 181.95 (C4); ESI-MS: *m/z* 287 $[M+H]^+$. Anal. calcd for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52; found: C, 62.92; H, 3.53%.

7-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-5,6-dihydroxy-4H-chromen-4-one **(6)**: Benzyl bromide (0.13 mL, 1.1 mmol, 2.2 equiv.) and K_2CO_3 (166 mg, 1.20 mmol, 2.4 equiv.) were added to a stirred solution of **2** (143 mg, 0.50 mmol) in dry acetone (20 mL), and this mixture was refluxed for 6 h. The reaction mixture was cooled to room temperature, and filtered and then concentrated under vacuum. The crude material was purified by chromatography on silica gel column using 25% ethyl acetate in petroleum ether afforded **6** (125 mg, 53.8% yield) as yellow solid; m.p. 139–141 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.03 (s, 2H, –OCH_2), 5.23 (s, 2H, –OCH_2), 6.62 (s, 1H, C3H), 6.87 (s, 1H, C8H), 7.18 (d, 2H, *J* = 8.6 Hz, C3′,C5′H), 7.31–7.53 (m, 10H, -Ph), 8.04 (d, 2H, *J* = 8.6 Hz, C2′,C6′H), 10.82 (s, 1H, C6OH), 13.11 (s, 1H, C5OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 73.93 (C($PhCH_2$)), 75.16 (C($PhCH_2$)), 98.44 (C8), 102.52 (C3), 103.94 (C10), 116.02 (C3',C5'), 121.03 (C1'), 127.50 (C6), 127.58 (PhC), 127.62 (PhC), 127.84 (2×PhC), 127.92 (PhC), 127.99 (2×PhC), 128.03 (PhC), 128.04 (2×PhC), 128.29 (2×PhC), 128.34 (C2',C6'),148.89 (C9), 157.03 (C5), 157.29 (C7), 161.18 (C4'), 163.99 (C2), 181.72 (C4); ESI-MS: *m/z* 465 $[M-H]^-$. Anal. calcd for $C_{29}H_{22}O_6$: C, 74.67; H, 4.75; found: C, 74.68; H, 4.72%.

7-(Benzyloxy)-2-(4-(benzyloxy)phenyl)-5-hydroxy-6-methoxy-4H-chromen-4-one **(7)**: Iodomethane (0.014 mL, 0.22 mmol, 1.2 equiv.) and K_2CO_3 (48 mg, 0.35 mmol, 1.4 equiv.) were added to a stirred solution of **6** (84 mg, 0.18 mmol) in dry DMF (20 mL) at room temperature. This mixture was allowed to react for 12 h, and then between 100 mL water and 100 mL ethyl acetate. Then the ethyl acetate layer was washed with 100 mL brine, dried over Na_2SO_4, filtered and concentrated under vacum. The crude material was purified by chromatography on silica gel column using 25% ethyl acetate in petroleum ether to afford **7** (81 mg, 94.0% yield) as a yellow solid; m.p. 196–198 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.00 (s, 3H, –OCH_3), 4.95 (s, 2H, –OCH_2), 5.28 (s, 2H, –OCH_2), 6.62 (s, 1H, C3H), 6.94 (d, 2H, *J* = 8.6 Hz, C3′,C5′H), 7.00 (s, 1H, C8H), 7.32–7.42 (m, 6H, -Ph), 7.44–7.54 (m, 4H, -Ph), 7.94 (d, 2H, *J* = 8.6 Hz, C2′,C6′H), 12.69 (s, 1H, C5OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.42 (C(CH_3)), 70.41 (C($PhCH_2$)), 75.15 (C($PhCH_2$)), 97.02 (C8), 103.19 (C3), 103.97 (C10), 114.39 (C3',C5'), 122.64 (C1'), 122.84 (C6), 127.57 (PhC), 127.84 (PhC), 127.94 (2×PhC), 128.06 (PhC), 128.14 (2×PhC), 128.30 (PhC), 128.33 (2×PhC), 128.36 (2×PhC), 128.36 (C2',C6'), 148.91 (C9), 156.47 (C5), 157.36 (C7), 162.28 (C4'), 163.41 (C2), 181.93 (C4); ESI-MS: *m/z* 481 $[M+H]^+$. Anal. calcd for $C_{30}H_{24}O_6$: C, 74.99; H, 5.03; found: C, 75.01; H, 5.04%.

5,7-Dihydroxy-2-(4-hydroxyphenyl)-6-methoxy-4H-chromen-4-one **(3)**: After 10% Pd/C (2 mg) was added to a solution of **7** (100 mg, 0.21 mmol) in ethanol (10 mL) and THF (10 mL), the atmosphere over reaction mixture was then replaced by hydrogen. The mixture was filtered after it was reacted for 8 h, the filtrate was concentrated and the crude material was purified by chromatography on silica gel column using 50% ethyl acetate in petroleum ether to afford **3** (60 mg, 96.1%) as yellow solid; m.p. 279–282 °C (lit.[18] 281–283 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.72 (s, 3H, –OCH_3), 6.58 (s, 1H, C3H), 6.89 (d, 2H, *J* = 8.6 Hz, C3′,C5′H), 7.11 (s, 1H, C8H), 7.87 (d, 2H, *J* = 8.6 Hz, C2′,C6′H), 10.19 (s, 1H, C4′OH), 10.72 (s, 1H, C7OH), 12.76 (s, 1H, C5OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.48 (C(CH_3)), 98.65 (C8), 102.99 (C3), 103.31 (C10), 114.44 (C3',C5'), 127.74 (C2',C6'), 128.55 (C1'), 153.05 (C6), 153.46 (C9), 162.24 (C5), 163.07 (C7), 173.10 (C4'), 173.14 (C2), 182.06 (C4); ESI-MS: *m/z* 299 $[M-H]^-$. Anal. calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03; found: C, 64.02; H, 4.01%.

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