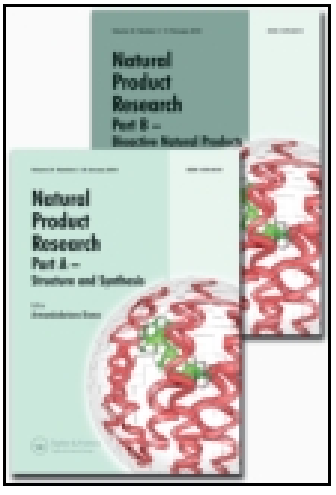

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Efficient synthesis of scutellarein

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Scutellarein is a component of *Scutellaria*, recently known as a potent cytotoxic agent on human leukaemia cells. The aim of this study was the synthesis of scutellarein and its methylated derivative. The new features are the innovating method to afford flavones from flavanones and the A-ring regioselective bromination step that lead to the target molecule by a facile and high-yielding pathway.

Keywords: scutellarein; flavonoids; methanolysis; polyphenols

1. Introduction

Flavonoids are a group of plant-derived compound that can be found in considerable quantities in fruits and vegetables (Beecher, 2003; Ross & Kasum, 2002). They exert antioxidant and biological activities due to their aromatic moieties and the presence of oxygenated groups (Fernandez-Bolanos, Felizon, Brenes, Guillen, & Heredia, 1998). The increasing interest in this kind of molecules is justified also by their low toxicity, which allows their use as drugs or food preservatives. Moreover, flavonoids have been shown to possess anticancer activity and flavonoid-based anticancer therapies are under development (Galati & O'Brien, 2004). Recently, scutellarein (Figure 1), a flavone extract from *Scutellaria*, became noteworthy due to its interesting biological activities reported by different research groups like cytotoxic activity on human leukaemia cells (Plochmann et al., 2007) and inhibitory activity towards 17β-HSD (Brozic et al., 2009) and human salivary α-amylase (Lo Piparo et al., 2008). It is also known to be an antagonist of tromboxane A_2 receptor (Navarro-Nuñez et al., 2009) leading to the formulation of new drugs involved in the thrombotic diseases. To the best of our knowledge, no synthesis of scutellarein was reported until now for preparative purposes (Gao & Kawabata, 2004). Therefore, we investigated a practical and high-yielding synthesis of scutellarein and its methylated derivatives in order to make them available to a deeper inspection.

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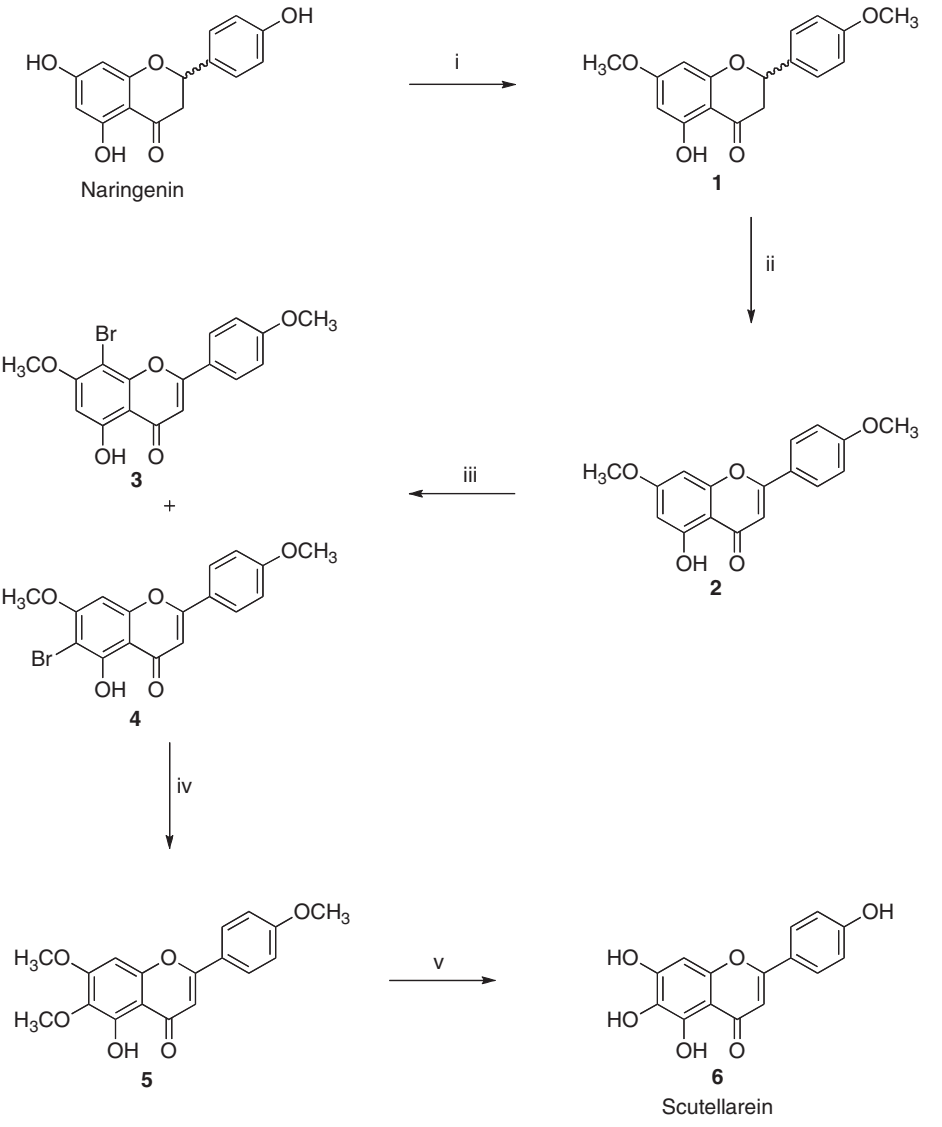

Figure 1. Convergent synthesis of scutellarein: (i) acetone, K_2CO_3, $(CH_3O)_2SO_2$; (ii) NMO, IBX, DMSO; (iii) TBATB, $CHCl_3$; (iv) CuBr, MeONa, DMF and (v) HBr, AcOH. Overall yield is 62% from naringenin.

2. Results and discussion

Our approach to the synthesis of scutellarein consisted in finding out a new and easily reproducible way to prepare it starting from inexpensive compounds. Referring to our previous work (Righi et al., 2010), in which crysin was converted to baicalein using a bromination/methanolysis protocol, the dimethylated apigenin (**2**) was chosen as starting material. Apigenin being very expensive and scarcely available in nature, our first goal was to convert naringenin, the flavanone precursor, into the above corresponding flavone. It is well known in literature that flavones

have enhanced biological activities than flavanones (Ares et al., 1996; Farkas, Jakus & Heberger, 2004; Park, 2004; Tapas, Sakarkar & Kakde, 2008). The oxidation step was carried out with the complex *N*-methylmorpholine-*N*-oxide (NMO) · 2-iodoxybenzoic acid (IBX) in DMSO at room temperature (RT; Figure 1). This procedure, reported few years ago by Nicolau, Montagnon, and Baran (2002), was recently applied on similar substrates (Barontini, Bernini, Crisante, & Fabrizi, 2010). To convert naringenin into **2**, the previous methylation of hydroxyl groups (Jurd, 1962) was necessary to avoid chemoselective issues arising from the presence of free hydroxyl groups since it is known that IBX can oxidise the B ring by introducing a second hydroxy group (Selenski & Pettus, 2006). The following bromination step led to a mixture of regioisomer, i.e. 6-bromo-5-hydroxy-7-methoxyflavone **3** and 8-bromo-5-hydroxy-7-methoxyflavone **4**, in accordance with our previous work. Tetrabutylammonium tribromide (TBATB) demonstrated to have a perfect regioselectivity towards the A ring since no bromination occurred on B ring. The mixture of monobromoflavones was directly converted to 6,7,4′-trimethoxyflavone (**5**) with copper bromide and sodium methoxide in DMF. A Wessely–Moser rearrangement occurs in basic media during the methanolysis step, as reported for crysin (Righi et al., 2010). The final selective demethylation steps led to scutellarein (**6**) passing through the methyl derivatives. The proposed synthesis occurred in 62% overall yield, allowing preparation of the target compound for drug uses and for extensive biological studies.

3. Experimental

3.1. *General*

NMR spectra were recorded on a Varian Mercury 3000 instrument (^{1}H 300 MHz, ^{13}C 75 MHz). Chemical shifts were calculated from the residual solvent signals of chloroform-d (^{1}H-NMR $\delta = 7.26$ ppm, ^{13}C-NMR $\delta = 77.0$ ppm) and DMSO-d_6 (^{1}H-NMR $\delta = 2.50$ ppm, ^{13}C-NMR $\delta = 77.0$ ppm). Melting points were measured on a Mettler FP80 instrument and were uncorrected. HRMS were performed on a Q-TOF MICRO spectrometer (Micromass, now Water, Manchester, UK) equipped with an ESI source. All chromatographic purifications were performed on silica gel (100–200 mesh from E. Merck, Germany). Thin-layer chromatography was performed on precoated silica gel 60 F_{254} aluminium sheets (Merck Italia) and spots were visualised under UV torch. All reagents used were of analytical grade and were purchased from Aldrich Chemical Co. Organic solvents used for the chemical synthesis and for chromatography acquired from Merck Italia were of analytical grade.

3.1.1. *7,4′-Dimethylnaringenin (**1**)*

Potassium carbonate (507 mg, 3.67 mmol) and dimethyl sulphate (0.35 mL, 3.67 mmol) were added to a solution of naringenin (500 mg, 1.84 mmol) in acetone (15 mL). The reaction mixture was stirred at 50°C overnight, and then aqueous NH_3 (3 mL) was added to quench the reaction. The acetone was evaporated under vacuum and HCl 2N was added in order to obtain an acidic solution. The resulting aqueous phase was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was

washed with brine (3×10 mL) and dried over anhydrous Na_2SO_4 and the solvent was evaporated to obtain a yellow powder. The crude product was purified over silica gel (eluent: hexane/ethyl acetate 7/3) to afford **1** (498 mg, 1.66 mmol, 90% yield) as yellow needles, m.p. 113.8–114.5°C (literature 114–115°C) (Oyama & Kondo, 2004). ^{1}H-NMR (chloroform-d_1) δ (ppm): 2.774 (dd, 1H, C^3–H, $J = 3$ Hz, $J = 17.1$ Hz), 3.096 (dd, 1H, C^3–H, $J = 12.9$ Hz, $J = 17.1$ Hz), 3.797 (s, 3H, CH_3), 3.828 (s, 3H, CH_3), 5.355 (dd, 1H, C^2–H, $J = 12.9$ Hz, $J = 3$ Hz), 6.035 (d, 1H, CH, $J = 2.4$ Hz), 6.064 (d, 1H, CH, $J = 2.4$ Hz), 6.950 (d, 2H, CH, $J = 8.7$ Hz), 7.378 (d, 2H, CH, $J = 8.7$ Hz) and 12.040 (s, 1H, OH); ^{13}C-NMR (chloroform-d_1) δ (ppm): 43.1 (CH_2), 55.3 (OCH_3), 55.6 (OCH_3), 79.0, 94.2, 95.0, 103.1, 114.2, 127.7, 130.4, 160.0, 162.9, 164.1, 167.9 and 195.9 (C=O). HRMS: calcd for $C_{17}H_{16}O_5Na^+$ ($M + Na^+$) 323.0890; found 323.0873.

3.1.2. *7,4′-Dimethylapigenin (**2**)*

N-methylmorpholine-*N*-oxide (123 mg, 1.09 mmol) was added to a solution of IBX (222 mg, 1.09 mmol) in DMSO (0.8 mL). When a clear solution was obtained (about 15 min), **1** (100 mg, 0.33 mmol) was added and the reaction mixture was stirred for 48 h at RT. The mixture was then diluted with ethyl acetate (5 mL) and the organic layer was sequentially washed with $NaHCO_3$ saturated solution (2×3 mL) and brine (3 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to give a yellow powder. The crude product was purified over silica gel (eluent: hexane/ethyl acetate 8/2) to afford **2** (89 mg, 0.30 mmol, 92% yield) as yellow needles, m.p. 169.1–172.2°C, (literature 167–170°C) (Areche, Schmeda-Hirschmann, Theoduloz, & Rodriguez, 2009). ^{1}H-NMR (chloroform-d_1) δ (ppm): 3.852 (s, 3H, OCH_3), 3.866 (s, 3H, OCH_3), 6.323 (d, 1H, CH, $J = 2.4$ Hz), 6.434 (d, 1H, CH, $J = 2.4$ Hz), 6.523 (s, 1H, C^3–H), 6.973 (d, 2H, CH, $J = 9$ Hz), 7.790 (d, 2H, CH, $J = 9$ Hz) and 12.783 (s, 1H, OH); ^{13}C-NMR (chloroform-d_1) δ (ppm): 55.7 (OCH_3), 55.9 (OCH_3), 92.8, 98.2, 104.5, 105.7, 114.7, 128.2, 157.9, 162.4, 162.8, 164.2, 165.6 and 182.6 (C=O). HRMS: calcd for $C_{17}H_{14}O_5Na^+$ ($M + Na^+$) 321.0733; found 321.0758.

3.1.3. *6-Bromo-5-hydroxy-7,4′-dimethoxyflavone (3) and 8-Bromo-5-hydroxy-7, 4′-dimethoxyflavone (**4**)*

TBATB (133 mg, 0.30 mmol) was added to a solution of **2** (89 mg, 0.30 mmol) in chloroform (3 mL). The solution was stirred for 80 min at RT and then the reaction was diluted with ethyl acetate (6 mL). The organic layer was washed with brine (3×5 mL) and dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to afford a 2:1 mixture of **3** and **4** (105 mg, 0.28 mmol, 94% yield). The mixture was used in the following step without any further purification. Compounds **3** and **4** were characterised after column chromatography on silica gel (eluent: hexane/ethyl acetate 8/2). Compound **3** (61 mg, 0.16 mmol) m.p. 248.1–249.5°C (recrystallised from acetone). ^{1}H-NMR (chloroform-d_1) δ (ppm): 3.872 (s, 3H, OCH_3), 4.001 (s, 3H, OCH_3), 6.549 (s, 1H), 6.614 (s, 1H), 7.019 (d, 2H, CH, $J = 6.6$ Hz), 7.820 (d, 2H, CH, $J = 6.6$ Hz) and 12.357 (s, 1H, OH). ^{13}C-NMR (chloroform-d_1) δ (ppm): 55.5 (OCH_3), 56.8 (OCH_3), 90.7, 95.9, 104.2, 105.8, 114.5,

123.0, 123.2, 128.3, 128.4, 153.2, 158.2, 161.5, 162.7, 164.1 and 181.7 (C=O); HRMS: calcd for $C_{17}H_{13}BrO_5Na^+$ ($M + Na^+$) 398.9839; found 398.9868.

Compound **4** (24 mg, 0.06 mmol) m.p. 248.3–249.7°C, (literature 249–250°C) (D.J. Donnelly, J.A. Donnelly, & Philbin, 1972). ^{1}H-NMR (chloroform-d_1) δ (ppm): 3.904 (s, 3H, OCH_3), 3.979 (s, 3H, OCH_3), 6.432 (s, 1H), 6.607 (s, 1H), 7.002 (d, 2H, CH, J°=°6.6°Hz), 7.933 (d, 2H, CH, J°=°6.6°Hz) and 12.145 (s, 1H, OH); ^{13}C-NMR (chloroform-d_1) δ (ppm): 55.5 (OCH_3), 56.7 (OCH_3), 88.0, 94.2, 103.5, 103.6, 114.7, 122.9, 123.1, 128.0, 128.4, 156.5, 161.3, 161.6, 162.8, 164.2 and 182.2 (C=O); HRMS: calcd for $C_{17}H_{13}BrO_5Na^+$ (M°+°Na^+) 398.9839; found 398.9882.

3.1.4. *6,7,4′-Trimethylscutellarein (**5**)*

DMF (0.7 mL) was added to a suspension of CuBr (30 mg, 0.21 mmol) in a 25% solution of sodium methoxide in methanol (1.89 mL, 8.32 mmol) and left under stirring at RT until a bright blue colour appeared (about 1 h). The mixture was added to a solution of **3** and **4** (96 mg, 0.26 mmol) dissolved in DMF (2.1 mL) at 120°C in 0.5 mL portion. The mixture was left stirring for 5 h and then cooled down to RT and washed with cold HCl 2N (3 mL). The reaction mixture was extracted with diethyl ether (3 × 10 mL) and the combined organic layer was washed with brine (3 × 10 mL) and dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The crude product was purified over silica gel (eluent: hexane/ethyl acetate 8/2) to afford **5** (70 mg, 0.21 mmol, 82% yield) as pale yellow needles, m.p. 180.3–181.1°C, (literature 180°C) (Achari, Chaudhuri, Saha, Dutta, & Pakrashi, 1990). ^{1}H-NMR (chloroform-d_1) δ (ppm): 3.866 (s, 3H, OCH_3), 3.901 (s, 3H, OCH_3), 3.948 (s, 3H, OCH_3), 6.525 (s, 1H), 6.577 (s, 1H), 6.980 (d, 2H, CH, $J = 8.1$ Hz), 7.809 (d, 2H, CH, $J = 8.1$ Hz) and 12.325 (s, 1H, OH); ^{13}C-NMR (chloroform-d_1) δ (ppm): 55.5 (OCH_3), 56.3 (OCH_3), 60.8 (OCH_3), 90.6, 104.2, 106.2, 114.5, 123.6, 128.0, 132.7, 153.1, 153.2, 158.7, 162.6, 164.0 and 182.7 (C=O). HRMS calcd for $C_{18}H_{16}O_6Na^+$ ($M + Na^+$) 351.0839; found 351.0798.

3.1.5. *Scutellarein (**6**)*

A solution of bromidic acid in water 47% (2.5 mL) was added to a solution of **5** (70 mg, 0.21 mmol) in glacial acetic acid (5.1 mL) at reflux. The solution was stirred for 72 h and then quenched with ice (100 g). The yellow precipitate was collected after filtration under vacuum and dried in oven (60°C) overnight. Compound **6** (58 mg, 0.20 mmol, 97% yield) was obtained as a yellow powder. Analytical data agreed with those reported in literature (Gao & Kawabata, 2004).

4. Conclusions

In this study, scutellarein and its 6,7,4′-methylated derivate were synthesised with an easy and high-yielding route by applying our previous procedure. These results highlighted the versatility of our semi-synthesis approach to the preparation of flavonoids. The presence of an activated B ring will allow further modification, which will be the next goal of our research group.

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