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Efficient synthesis of polyoxygenated flavones from naturally occurring flavanones

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Abstract

Flavonoids are constituents of the human diet (they are present in many beverages and food), and in organisms they are responsible for several biological functions, including that of antioxidant. Because of the increasing interest in these molecules, methods for their synthesis and structural modification are of great importance; studies on the biological activities of many of these compounds are insufficient because of their scarcity and/or high cost. We have developed an expeditious synthesis of polyoxygenated flavones, starting from available and inexpensive flavanones, using a bromination–methoxylation procedure. A series of flavonoids that are not otherwise accessible can be prepared using this method. As an example, 3′-demethoxysudachitin, a limited flavone possessing antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori* and acting as a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger, was prepared in fairly satisfactory yield.

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Introduction

Natural antioxidants have been isolated from various plants. They are usually polyphenols, the most important of which are flavonoids, having a benzopyrane skeleton. Flavones, which have an unsaturated C-ring, and flavanones, possessing a saturated C-ring, represent typical examples of this class of compounds.

Flavonoids occur widely in the plant kingdom, especially in fruits and vegetables, and they are the most abundant polyphenols in the human diet. Many flavonoids, including several hydroxyflavones and flavanones, have been shown to possess free-radical scavenger properties (Cotelle et al 1996), and are therefore possible candidates for the treatment of diseases that involve oxidation processes.

Methoxylated flavones that exhibit interesting biological activities have recently been isolated from plants, for example nevadensin (5,7-dihydroxy-6,8,4′-trimethoxyflavone), which has antimycobacterial activity, and isothymusin (6,7-dimethoxy-5,8,4′-trihydroxyflavone), which has antioxidant activity (Aroonrerk et al 2003). A semisynthetic analogue of fumagillin (TNP-470) has been prepared and has been shown to inhibit endothelial cell proliferation by interfering with endothelial cell DNA synthesis (Bernsen & van der Kogel 1999). Similarly, flavopiridol, a semisynthetic flavone analogue of natural rohitukine, inhibits several cyclin-dependent kinases (CDKs) and is the first CDK inhibitor to be tested in human clinical trials for the treatment of cancer and proliferative disorders (Senderowicz 1999, 2001).

Despite the biological importance evident for this class of natural compounds, extensive investigation into the activities of most of them is limited because of their scarcity. Moreover, the synthesis and/or structural modification of these polyphenols is often complex, low yielding and expensive (Stermitz et al 1975; Sanicanin & Tabakovic 1986; Matuyama et al 1989).

Material and Methods

Each synthetic step was repeated in triplicate or until results were reproduced with a maximum deviation of 5% with respect to that reported in the experimental procedures.

General procedure for the bromination of flavanones

To a 0.01 M solution of the appropriate flavanone and NaBr (1 equivalent for every bromine atom to be introduced in the substrate) in a 1:1 mixture of acetone and water, oxone was added in small portions until consumption of the substrate (usually 1.4 eq.); the course of the reaction was monitored by thin-layer chromatography (TLC) (hexane/EtOAc 1:1). $Na_2S_2O_3$ was added to reduce the excess of oxone, until no reaction of a NaI solution occurred. The mixture was then extracted twice with EtOAc. The organic extracts were washed with a saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4 and the solvent evaporated in vacuum.

*6,8-Dibromo-5-methoxyflavanone (**2**)*
We used 250 mg (1 mmol) 5-methoxyflavanone, 204 mg (2 mmol) NaBr and 1.85 g (1.5 mmol) oxone, and the reaction was complete in 30 min. After flash chromatography (hexane/EtOAc 8:2), 410 mg (90%) of product were obtained as brown oil. ^{1}H-NMR ($CDCl_3$) δ(ppm): 3.02 (1H, d, J = 4.5 Hz), 3.06 (1H, d, J = 11.3 Hz), 3.90 (3H, s), 5.56 (1H, dd, J = 4.5, 11.3 Hz), 7.43 (5H, m), 7.92 (1H, s). ^{13}C-NMR ($CDCl_3$) δ(ppm): 44.9, 61.7, 79.3, 107.4, 110.7, 117.1, 125.8, 128.8, 128.9, 137.7, 137.6, 141.2, 156.7, 158.1, 188.6. Anal. calcd for $C_{16}H_{12}Br_2O_3$: C, 46.64; H, 2.94. Found: C, 46.40; H, 2.82.

*6-Bromoflavanone (**10**)*
The product was obtained by the above procedure from flavone in 96% yield. Physical data were in accordance with that reported in literature (Ramadas & Krupadanam 2004).

*8-Bromo-5,7,4′-trimethoxyflavanone (**12**)*
We used 200 mg (0.64 mmol) naringenin trimethyl ether, 144 mg (1.4 mmol) NaBr and 1.23 g (1 mmol) oxone. After 45 min the mixture was extracted as usual and the product purified by flash chromatography (hexane/EtOAc 8:2) to yield a yellow–brown oil. ^{1}H-NMR ($CDCl_3$) δ(ppm): 2.93 (1H, d, J = 4.0 Hz), 2.99 (1H, d, J = 11.3 Hz), 3.81 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 5.48 (1H, dd, J = 4.0, 11.3 Hz), 6.15 (1H, s), 6.93 (1H, d, J = 8.8 Hz), 7.42 (1H, d, J = 8.8 Hz). ^{13}C-NMR ($CDCl_3$) δ(ppm): 44.9, 55.3, 56.2, 56.4, 78.8, 89.4, 91.7, 106.4, 114.1, 127.3, 130.5, 159.8, 160.1, 161.6, 161.9, 189.1. Anal. calcd for $C_{18}H_{17}BrO_5$: C, 54.98; H, 4.36. Found: C, 54.65; H, 4.25.

*6,8-Dibromo-5,7,4′-trihydroxyflavanone (**17**)*
We used 250 mg (0.92 mmol) naringenin, 204 mg (2 mmol) NaBr and 1.48 g (1.2 mmol) oxone. After 30 min, a non-crystallizable yellow solid separated. The filtered product (336 mg, 85%) was shown to be pure by NMR analysis. ^{1}H-NMR (acetone-d_6) δ(ppm): 2.95 (1H, dd, J = 12.2, 17.1 Hz), 3.33 (1H, dd, J = 3.4, 17.1 Hz), 5.66 (1H, dd, J = 3.4, 12.7 Hz), 6.91 (2H, d, J = 8.8 Hz), 7.43 (2H, d, J = 8.8 Hz), 13.01 (1H, s). ^{13}C-NMR (acetone-d_6) δ(ppm): 80.6, 90.1, 91.1, 103.8, 116.3, 128.9, 130.0, 158.9, 159.2, 160.1, 160.2, 197.4. Anal. calcd for $C_{15}H_{10}Br_2O_5$: C, 41.89; H, 2.34. Found: C, 41.65; H, 2.15.

Typical procedure for the oxidation of flavanones to flavones

The appropriate flavanone (0.45 mmol) was dissolved in DMSO (1 mL) and the solution heated to 100°C. To the warm solution were added catalytic amounts of I_2 (10 mg, 0.05 mmol) and concentrated H_2SO_4 (1 drop), and the reaction was monitored by TLC (hexane/EtOAc). When the substrate was completely consumed, the mixture was quenched with ice and a 0.1 M aqueous solution of $Na_2S_2O_3$ added to destroy the I_2 residue. The mixture was extracted with EtOAc (3 × 10 mL) and the reunited organic phases washed with a saturated aqueous NaCl solution (2 × 5 mL). The organic solution was then dried over anhydrous Na_2SO_4 and the solvent evaporated under vacuum. Products that did not need any further chromatographic purification were obtained in good yields (73% to > 95%, Table 1).

Data for compounds **6** (Moon et al 2005), **8** (Shen et al 1993), **9** (Rho et al 2002), **11** (Marder et al 1996) and **13** (Khan & Goswami 2005) were in accordance with that reported in literature.

*6,8-Dibromo-5-methoxyflavone (**3**)*
185 mg (0.45 mmol) of 6,8-bromo-5-methoxyflavanone gave 135 mg (73%) of product as a brown oil. ^{1}H-NMR ($CDCl_3$) δ(ppm): 3.98 (3H, s), 6.81 (1H, s), 7.54–7.57 (3H, m), 7.97–8.02 (2H, m), 8.11 (1H, s). ^{13}C-NMR ($CDCl_3$) δ(ppm): 62.0, 105.7, 107.4, 108.3, 114.6, 129.2, 132.0, 132.7, 139.5, 155.8, 161.9, 176.2. Anal. calcd for $C_{16}H_{10}Br_2O_3$: C, 46.86; H, 2.46. Found: C, 46.50; H, 2.2.

*6,8-Bromo-5,7,4′-trihydroxyflavone (**18**)*
300 mg of **17** (0.7 mmol) gave 275 mg (92%) of a yellow solid that was difficult to manipulate because of its low

Table 1 Oxidation of flavanones to flavones

Substrate	Time (min)	Product	Yield (%)
6-OMe-flavone (**1**)	20	6-OMe-flavone (**9**)	92
6,8-Br-5-OMe-flavanone (**2**)	40	6,8-Br-5-OMe-flavone (**3**)	73
Flavanone (**5**)	15	Flavone (**6**)	95
Naringenin (**7**)	90	Apigenin (**8**)	95
6-Br-flavanone (**10**)	30	6-Br-flavone (**11**)	90
8-Br-5,7,4′-OMe-flavanone (**12**)	50	8-Br-5,7,4′-OMe-flavone (**13**)	90
6,8-Br-5,7,4′-OH-flavanone (**17**)	60	6,8-Br-5,7,4′-OH-flavone (**18**)	92

solubility in most solvents. It was characterized as triacetate. Crude product (275 mg) was treated with 1 mL pyridine and 1 mL acetic anhydride and left overnight. The mixture was quenched with a 1 M solution of HCl, the solution extracted with EtOAc (3 × 10 mL) and the organic solution washed to neutrality with a saturated aqueous NaCl solution. The solution was then dried over anhydrous Na_2SO_4 and the solvent evaporated under vacuum. A yellow-brown oil was obtained. ^{1}H-NMR ($CDCl_3$) δ(ppm): 2.33 (3H, s), 2.47 (3H, s), 2.49 (3H, s), 6.70 (1H, s), 7.26 (1H, d, J = 8.8 Hz), 7.96 (1H, d, J = 8.8 Hz). ^{13}C-NMR ($CDCl_3$) δ(ppm): 20.4, 20.8, 21.1, 105.8, 108.1, 110.9, 116.7, 122.5, 127.8, 147.1, 150.7, 153.2, 153.7, 161.8, 166.1, 167.9, 168.7, 175.0, 183.4. Anal. calcd for $C_{21}H_{14}Br_2O_8$: C, 45.52; H, 2.55. Found: C, 45.30; H, 2.22.

Typical procedure for bromo-flavones methanolysis

The protocol was improved with respect to that previously reported (Bovicelli et al 2006). A 25% solution of MeONa in MeOH (7.94 mL, 35 mmol, 4 eq. for every bromine atom to substitute) was mixed at room temperature with CuBr (125 mg, 0.87 mmol) and DMF (0.8 mL). After a few minutes' stirring, a blue solution was formed, which was added dropwise over 1 h to a solution of dibromoflavanone (4.37 mmol) in DMF (6.7 mL) stirred at 120°C. The mixture was then quenched with ice and extracted with EtOAc (3 × 50 mL). The organic layers were dried over anhydrous Na_2SO_4 and the solvent evaporated.

*5,6,8-Trimethoxyflavone (**4**)*

410 mg (1 mmol) of 5-methoxy-6,8-dibromoflavone **3** gave 278 mg (89%) of **4** as a colourless oil. Physical data were in accordance with that reported by Iinuma et al (1980).

*3′-Demethoxysudachitin (**19**)*

500 mg (1.14 mmol) of 6,8-dibromoapigenin **18** gave 300 mg (80%) of pure product as a colourless oil. 1H-NMR (acetone-d6) d: 3.87 (3H, s), 3.96 (3H, s), 6.65 (1H, s), 7.04 (2H, d, J=8.8 Hz), 7.97 (2H, d, J=8.8 Hz), 12.92 (1H, s). 13C-NMR (acetone-d6) d: 60.8, 61.8, 103.6, 104.6, 116.5, 117.0, 123.4, 129.2, 132.4, 146.7, 149.8, 151.3, 162.0, 165.0, 183.7. Anal. calcld for $C_{17}H_{14}O_7$: C, 61.82; H, 4.27. Found: C, 61.9; H, 4.5.

Results and Discussion

Recently, we optimized a method for introducing oxygenated moieties into activated aromatic rings, convenient to prepare polyphenols starting from inexpensive and easily available natural compounds. A selective bromination (Bovicelli et al 2001, 2002), followed by a methanolysis promoted by the system MeO^-/CuBr, allowed the synthesis of complex compounds such as ubiquinones (Bovicelli et al 2005), bioactive biphenols (Bovicelli et al 2006) and hydroxytyrosol (Bovicelli et al 2007). The methanolysis step is already known, but the procedures reported in the literature (Bacon & Rennison 1969) showed poor reproducibility, which is probably why this reaction is rarely used in synthesis and is not considered a general route for the poly-oxygenation of aromatics. While trying to improve the experimental procedure, we found a protocol that proved highly successful for many of the substrates we used (Bovicelli et al 2005). Unfortunately, however, the reaction failed when sensitive compounds such as flavones were submitted to the transformation. Pursuing our objective of introducing oxygenated functionalities into flavonoids, we tried to improve the method further and succeeded in designing a protocol that, so far, seems to be completely successful.

In the original procedure, a mixture of the catalyst and a solution of sodium methoxide in DMF were heated at 130°C and the substrate added in one portion. In improving the procedure, we found both the nature of the catalyst, CuBr, which has to be carefully recrystallized before use, and the composition of the solvent, which must contain a small percentage of water to work well, to be of paramount importance.

Our protocol was improved as follows. We observed that a blue mixture formed when NaOMe and CuBr were mixed in DMF before the addition of the substrate and assumed the formation of a reactive complex. At 130°C, DMF rapidly decomposes; most probably some of the decomposition products interfere in some way in the process to reduce yields. To avoid this, in the new protocol, the blue complex is prepared separately and added to the warm solution of the substrate in DMF.

In the light of this new procedure, the selective bromination of flavonoids, together with many other polyphenols, becomes more attractive, since the products are not only appealing as new ones but also as intermediates for higher oxygenated species.

The bromination reaction carried out on flavones led to the decomposition of the starting material because of the lability of the enonic double bond on the C ring. To overcome this problem it is necessary to work on flavanones and to put off the introduction of the double bond into ring C by an oxidative step. This oxidation, previously reported for a single case (Fatma et al 1984), was successful mainly for bromo-flavanones and proved ineffective in the case of most methoxy derivatives.

Thus the correct sequence for obtaining a polymethoxylated flavone is initial bromination of the flavanone, followed by oxidation to the flavone, and finally methanolysis (Figure 1). The same strategy was adopted for the preparation of 3′-demethoxysudachitin. In this case, a product with alternated hydroxy and methoxy groups was obtained (Figure 2).

This pathway is often found in natural antioxidants, and the synthesis reported herein is just one example, the approach being extendible to many other compounds. In all cases, highly oxygenated and potentially bioactive products (antioxidants, antibacterial, etc.) can be obtained.

3′-Demethoxysudachitin (**19**) is well known and is widely present in several plant extracts, including that of *Baccharis grisebachii* (Asteraceae) used in Argentinean traditional medicine as a digestive and to relieve ulcers. It exhibited the highest activity in inhibition of erythrocyte lipoperoxidation compared with other hydroxylated flavonoids (Tapia et al 2004). Flavone **19** was also found to possess antimicrobial

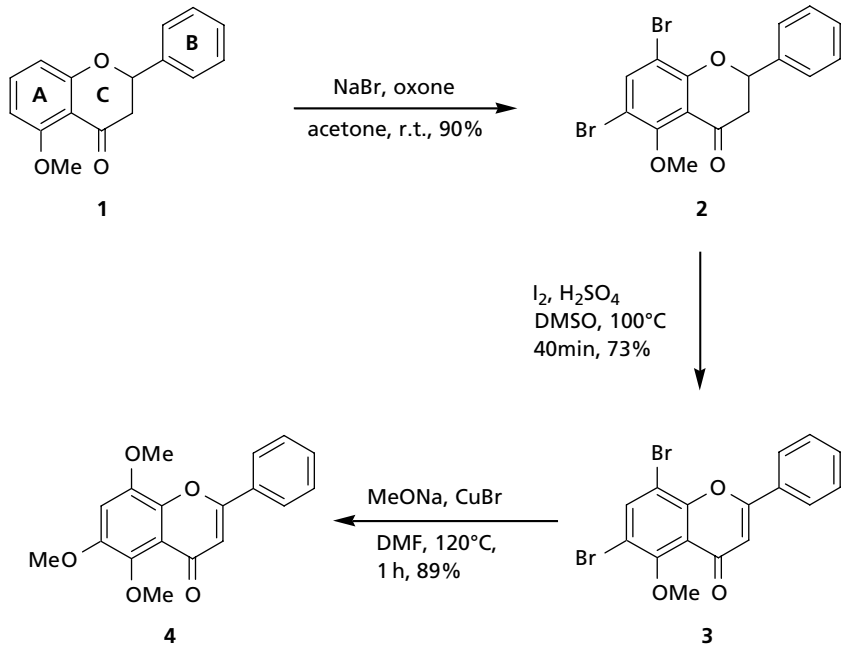

Figure 1 Synthesis of 5,6,7-trimethoxyflavone.

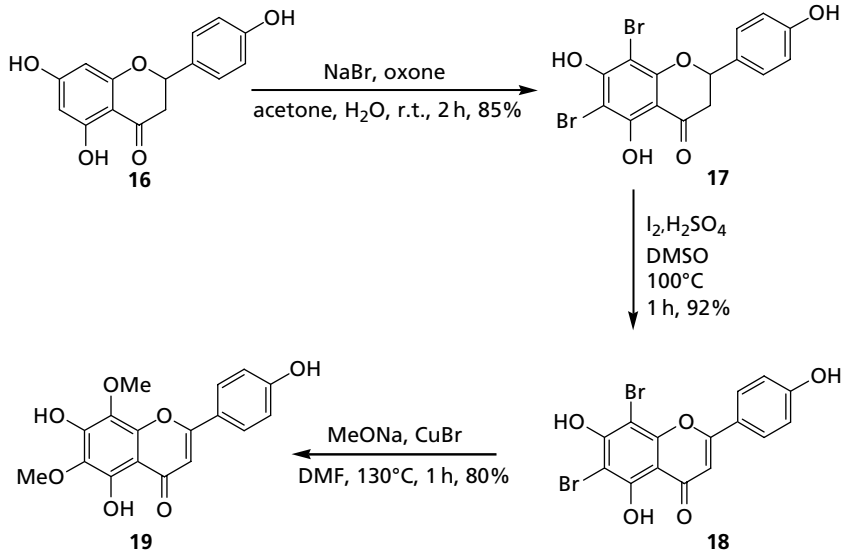

Figure 2 Synthesis of 6,8-dimethoxyapigenin.

activity against methicillin-resistant *Staphylococcus aureus* and *Helicobacter pylori* (Nakagawa et al 2006) and acts as a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger (Tapia et al 2004).

Conclusions

Investigations into the beneficial properties of flavonoids require a practical and easy method to prepare many compounds with many different functional groups. Some brominated flavonoids have been found to have biological properties, but the difficulties experienced in preparing derivatives of a large number of natural compounds has, to date, limited investigation. Polyoxygenated flavonoids were likewise not easily available until now. In this paper, we propose an efficient and easy transformation of simple and available flavanones into higher oxygenated flavones not easily obtainable by other methods. In particular, flavones highly oxygenated in ring A could be easily obtained, and many others can presumably be prepared by the same way.

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