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Synthesis and Rat Prostate 5-Alpha Reductase Inhibitions of Methylated Quercetin Derivatives

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Abstract

Interested in chemical and biological properties of polymethoxyflavones, a series of methylated quercetin derivatives were synthesized and characterized. Quercetin was methylated with CH_3I in the presence of K_2CO_3/DMF, and the products were purified by column chromatography. Three quercetin derivatives with different methyl group numbers were isolated and the chemical structures were confirmed by IR and NMR. Along with physical characterizations, rat prostate steroid 5α-reductase inhibition of the quercetin derivatives was measured. It was found that methylation of quercetin lowered the inhibitory effect of the derivatives against 5α-reductase.

Keywords: polymethoxyflavones, quercetin derivatives, steroid 5-alpha reductase

1. Introduction

Polymethoxyflavones (PMFs) are found from *Kaempferia parviflora* (1) and other *Citrus* plants (2). PMFs show various biological activities, including anticarcinogenic, anti-inflammatory, antioxidant, and antiviral activities (3-4).

Steroid 5α-reductase converts testosterone to dihydrotestosterone (DHT) (Figure 1). DHT functions as the primary androgen in the prostate and hair follicles. DHT is a critical mediator of prostatic growth and the primary contributing factor in male pattern baldness (5). Men with androgenic alopecia typically have higher 5α-reductase activity and lower total testosterone (6). Therefore, steroid 5α-reductase has been a target for the treatment of benign prostatic hyperplasia and androgenic alopecia (6-7).

Because various polyphenols and isoflavones are reported to have the ability to inhibit 5α-reductase activity (8-9), we have set out chemical derivatization of quercetin to test their inhibitory effects.

Here, preparation and 5α-reductase inhibition of three methylated quercetin derivatives were reported.

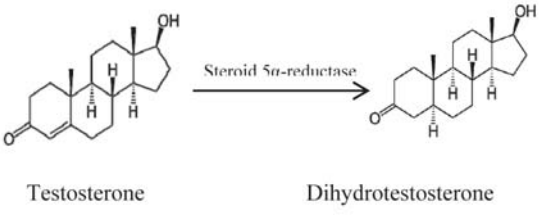


Figure 1. Conversion of testosterone to dihydrotestosterone (DHT)

2. Materials and Methods

2.1 Chemical synthesis

Quercetin was purchased from Alfa Aesar (Ward Hill, MA, USA) and other reagents were obtained from Samchun Chemicals (Pyeongtaek-si, Korea). DMF was purified and dried with CaH_2 prior to use. Reactions were monitored by thin-layer chromatography (TLC) using on Merck silica gel 60 F_{254} plates. TLC was performed with 1 : 1 mixture of hexanes and acetone or 1 : 1 mixture of hexanes and ethyl acetate. NMR spectra were recorded on a Varian Gemini 2000 (300 MHz). Compounds were dissolved in $CDCl_3$-*d* containing TMS as a reference. IR spectra of compounds in KBr pellet were obtained on a Shimadzu FT-IR 8400S spectrometer.

2.1.1 Synthesis of methylated quercetin derivatives 2, 3 and

To a solution of quercetin 1 (174 mg, 0.57 mmol) in dry DMF (20 ml) was added K_2CO_3 (416 mg, 3.0 mmol, 5.3 *eq*) and CH_3I (0.17 ml, 2.75 mmol, 4.8 *eq*) at room temperature. After 12h, the reaction mixture was poured into water (100 ml), and extracted with ethyl acetate (100 ml) three times. The combined extract was washed with brine (100 ml), dried over anhydrous $MgSO_4$, filtered through filter paper and concentrated (190 mg). The crude product was subjected to silica gel column chromatography (20% acetone in hexanes) to yield 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (2, 44.5 mg, 26%) as yellow powder, 5-hydroxy-3,7,3',4'-tetramethoxyflavone (3 ,85.5 mg, 48%) as yellow crystals, and 3,5,7,3',4'-pentamethoxyflavone (4, 15mg, 8%) as white solid.

Compound **2**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.80 (*s*, 6H, -OCH_3), 3.92 (*s*, 3H, -OCH_3), 5.64 (*s*, 1H, -OH), 6.29 (*d*, 1H, *J* = 2.2 Hz, 6-H), 6.37 (*d*, 1H, *J* = 2.2 Hz, 8-H), 6.91 (*d*, 1H, *J* = 5.5 Hz, 5'-H), 7.62 (*dd*, 1H, *J* = 2.2, 5.5 Hz, 6'-H), 7.66 (*d*, 1H, *J* = 2.2 Hz, 2'-H), 12.56 (*s*, 1H, 5-OH); IR, cm^{-1}: 3490 (OH), 1610 (C=O).

Compound **3**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.87 (*d*, -OCH_3), 3.88 (*d*, -OCH_3), 3.97 (*d*, -OCH_3), 3.88 (*d*, -OCH_3), 6.36 (*d*, 1H, *J* = 2.2 Hz, 6-H), 6.44 (*d*, 1H, *J* = 2.2 Hz, 8-H), 7.01 (*d*, 1H, *J* = 8.6 Hz, 5'-H), 7.69 (*d*, 1H, *J* = 2.0 Hz, 2'-H), 7.72 (*dd*, 1H, *J* = 2.0 Hz, 8.6 Hz, 6'-H), 12.56 (*s*, 1H, 5-OH); IR, cm^{-1}: 3447 (OH), 1602 (C=O).

Compound 4: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.87 (*s*, 3H, -OCH_3), 3.90 (*s,* 3H, -OCH_3), 3.96 (*s*, 9H, 3-OCH_3), 6.35 (*d*, 1H, *J* = 2.2 Hz, 6-H), 6.51 (*d*, 1H, *J* = 2.2 Hz, 8-H), 6.99 (*d*, 1H, *J* = 9.2 Hz, 5'-H), 7.69 (d*d*, 1H, *J* = 2.0 Hz, 9.2 Hz, 6'-H), 7.72 (*d*, 1H, *J* = 2.0 Hz, 2'-H); IR, cm^{-1}: 1624 (C=O).

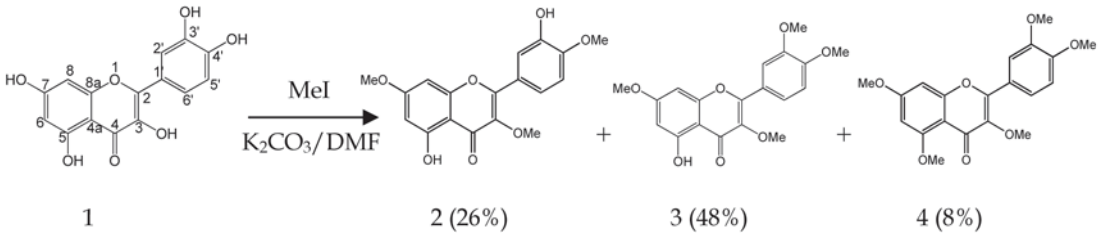
2.2 Steroid 5α-reductase inhibitions

The enzyme suspension of testosterone 5α-reductase was prepared from the homogenate of the ventral prostates of male Sprague-Dawley rats according to the previously reported method (10). The testosterone 5α-reductase inhibitory activity was also measured according to the previously reported method (10). Each enzyme reaction was carried out in duplicate, and the half maximum inhibitory concentration (IC_{50}) was calculated from the values of the inhibitory activities at two concentrations.

3. Results and Discussion

3.1 Synthesis

Quercetin (**1**) was methylated with methyl iodide in the presence of base to synthesize the methylated quercetin derivatives (Scheme 1) (11). From the reaction mixture, three methylated quercetin derivatives, 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (**2**), 5-hydoroxy-3,7,3',4'-tetramethoxyflavone (**3**), and 3,5,7,3',4'-pentamethoxyflavone (**4**) were purified by general silica gel column chromatography (Scheme 1). The isolation yields of three products were 26%, 48% and 8%, respectively.



Scheme 1. Synthesis of methylated quercetin derivatives

The chemical structures of the compounds were confirmed by IR and ^{1}H NMR spectroscopy. ^{1}H NMR spectrum of compound **2** showed two hydroxyl group peaks at 5.64 (3' position) and 12.56 (5 position) ppm. However, only one 5-hydroxyl group was found at 12.56 ppm in compound **3**. In the ^{1}H NMR spectrum of compound **4**, no hydroxyl group was observed and five methyl peaks were found at the region of 3.9 ppm. In the IR spectra of compound **2** and **3**, hydroxyl groups were observed at 3490 and 3447 cm^{-1}, respectively. The carbonyl groups of compound **2**, **3** and **4** were observed at 1610, 1602 and 1624 cm^{-1}, respectively.

3.2 Steroid 5α-reductase inhibitions

Quercetin and three synthesized methylated quercetin derivatives were evaluated for their inhibitory activity against steroid 5α-reductase. Riboflavin was included as a positive control. As shown at Table 1, the inhibitory effects of the compounds **2** and **3** were consistent with large data variations. Therefore, we discuss the inhibitory effect at only 100 μM. Overall, quercetin exhibited better inhibitory activity than the methylated derivatives. Besides, increased methylation seemed to lower the inhibitory effect.

In this study, three quercetin derivatives were synthesized and the inhibition effects of steroid 5α-reductase were examined.

From the comparison of structures and inhibitory effects of the PMFs, the flavone with more hydroxyl groups appears to increase the inhibitory activity. However, some inhibition percentages were measured over 100% at 1000 μM concentration, and the ranges of a few data were high. It could be poor solubility of methylated flavones or more likely lower activity of the prepared steroid 5α-reductase. Therefore, 5α-reductase inhibition test needs to be carried out again. Regardelss, a general tendency of methylation on the inhibition of 5α-reductase could be found with the limited number of data.

Table 1. Inhibitory effect of quercetin and the methylated derivatives on rat prostate testosterone 5α-reductase

Compound	Inhibition (%)		IC_{50} (μM)
	100 μM	1000 μM	
Quercetin (1)	95.6 ± 2.8	153.6 ± 44.4	16
5,3′-Dihydroxy-3,7,4′-trimethoxyflavone (2)	66.7 ± 7.6	95.4 ± 42.5	26
5-Hydroxy-3,7,3′,4′-tetramethoxyflavone (3)	36.6 ± 22.9	12.8 ± 41.3	> 1000
3,5,7,3′,4′-Pentamethoxyflavone (4)	48.2 ± 47.3	124.9 ± 3.3	110
Riboflavin	76.2 ± 6.8	96.2 ± 13.2	4.9

4. Acknowledgement

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