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Short Note

7-Docosahexaenoyl-Quercetin

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Abstract: Fatty acids and polyphenols represent different classes of pharmacologically active molecules. Hybrid derivatives of these compounds are interesting therapeutic tools. They could be obtained using enzymatic approaches, which allow regioselective derivatizations. In this short note, the pancreatic porcine lipase was employed to mediate the regioselective synthesis of 7-docosahexaenoyl-quercetin was described. The C-7 regioisomer formation was confirmed by ^{1}H-NMR experiment. Generally, in this approach the alcoholic OH- was preferred when present. Nevertheless, in this case, it was demonstrated that the hindrance of the acyl group is a variable to obtain a good regioselectivity in C-7 position, employing only one-step reaction.

Keywords: quercetin; DHA; lipase; regioselective; polyphenols

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1. Introduction

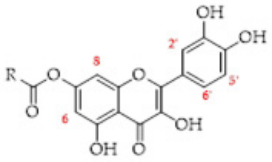
The free fatty acids are essential constituents of foods, plants, and humans. Most of them show important biological properties due to they are endogenous ligands of different biological targets [1]. In particular, the most famous fatty acids are polyunsaturated fatty acids (PUFA), those benefits are generally limited by their tendency to oxidation or lipotoxicity when used in high amount [2]. In order to limit metabolic features of PUFAs, emerging strategies involved their embedding to natural antioxidants [3]. A recent study reported how fish oil, constituted by high amounts of eicosapentaenoic and docosahexaenoic (DHA) acids, could be enriched with the flavonol quercetin that reacted with these PUFAs to form the corresponding C3-esters, limiting lipid oxidation [4]. Over the years, several synthetic strategies have been proposed to synthesize lipophilic and aromatic derivatives of polyphenols, especially flavonoids [5,6]. Nevertheless, classical chemical methods led to the formation of side products, limiting the yield and purity of final compounds. With the aim to recover good amounts of substances, some biocatalytic processes have been proposed. *Candida Antarctica B* (Novozyme 435®) seemed to be the frequently employed enzyme, able to promote the regioselective acylation of polyphenols and fatty acids in C-3 position of the chromene core. Although Novozyme 435® is very expensive, it is the most used in the acylation reactions of fatty acids with flavonoid glycosides [7]. Recently, this enzyme was also applied to prepare acylpolyphenols with fatty alcohols, providing interesting baicalin derivatives in high yield [8]. However, no reports are available for the synthesis of quercetin esters using Novozyme 435® [9]. Quercetin esters have been synthesized using different lipases [10]. In particular, quercetin-3-oleate was obtained by using Pancreatic Porcine Lipase (PPL) [11–13], capable to promote the C-3 acylation of quercetin. The enzyme occurred helpful for the preparation of C-3 oleoyl esters of other

polyphenols, including morin and catechin [14]. In the case of pinocembrin, C-7 acylation is favored with the representative oleic, linoleic, and linolenic acids [15]. In this context, we evaluated the use of docosahexaenoic acid (DHA) as acylating agent and PPL as catalyst for selective quercetin derivatization. This procedure demonstrates that, in the case of DHA, only the C-7 ester was obtained in good yield.

2. Results and Discussion

In our previous works, PPL was employed as acylating enzyme, providing the formation of C-3 and C-7 esters, although the C-3 position (bearing a vinylic or alcoholic hydroxyl) resulted preferred when present. In the same experimental conditions, we observed the only formation of C-7 acylated derivative by using DHA. This feature could be attributed to the hindered chain of DHA, that constituted a bulky activated intermediate that was not able to provide the C-3 acylation. In fact, the typical acylation mechanism starts with the formation of the enzyme-acyl intermediate that, in this case, is too bulky to interact with C-3 hydroxyl of the acyl acceptor (quercetin). The less hindered C-7 hydroxyl is available to interact with the intermediate, forming the corresponding enzyme-acyl-quercetin complex. Finally, this complex evolves through the formation of 7-docosanoylquercetin. The C-7 regioisomer formation was confirmed by ^{1}H-NMR experiment. Original spectra are available in Supplementary Materials. In particular, the ^{1}H resonances of the quercetin (Q) and the derivative (7-DQ) are reported:

Comparing these chemical shifts with the one of quercetin, it is possible to observe that the proton most affected by the esterification process is the one in position 8, with a decrease of about 0.40 ppm, while the other protons are the almost same (Figure 1). As reported by Mattarei et al. [16], this change could be attributed to an esterification occurring in position 7.



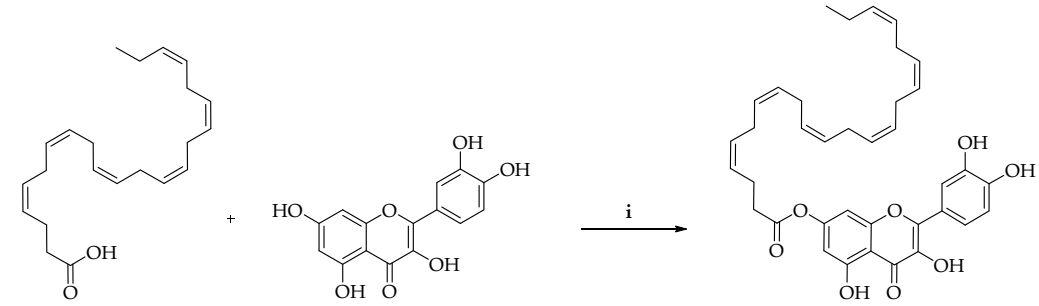
Position	δ ^{1}H (7-DQ)	δ ^{1}H (Q)
6	6.18	6.17
8	**6.40**	**6.77**
2′	7.68	7.68
5′	6.89	6.89
6′	7.54	7.54

Figure 1. Chemical shifts of the quercetin moiety of 7-docosahexanoylquercetin (7-DQ) and quercetin (Q).

3. Materials and Methods

The lipase from porcine pancreas (PPL), Type II, 100–500 units/mg protein (using olive oil (30 min incubation)), 30–90 units/mg protein (using triacetin), and Quercetin dehydrate ≥98% (HPLC), powder, and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) were purchased from Merck (Darmstad, Germania). Acetone was purchased from Levanchimica Srl (Bari, Italy). Quercetin ^{1}H NMR (400 MHz, DMSO-d_6): 7.68 (C-2″, d, J = 2.11 Hz, 1H); 7.55 (C-6″, dd, J = 2.11, 8.44 Hz, 1H); 6.89 (C-5″, d, J = 8.44 Hz, 1H); 6.77 (C-8′, s, 1H); 6.17 (C-6′, s, 1H). The selective C-7 position of acylation was determined by NMR. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Advance 400 spectrometer (Bruker, Milano, Italy) at 400 and 100 MHz, respectively. DMSO-d_6 was used as solvent for NMR experiment. ESI-MS spectrum was performed by an Agilent 1100 Series LC/MSD spectrometer operating in positive mode. Synthetic procedure (Figure 2) for the preparation of 7-docosanoylquercetin 2-(3,4-dihydroxyphenyl)-3,5-dihydroxy-4-oxo-4H-chromen-7-yl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate: In a round-bottom flask, to a solution of quercetin (100 mg, 0.330 mmol) and DHA (108.40 mg, 0.330 mmol) in 30 mL of acetone were added 260 mg of PPL. Temperature was maintained at 37 °C, with an agitation rate of 130 rpm. After 48 h, the reaction was monitored under UV light, after elution of a thin-layer chromatography (TLC) on silica gel 60 F254 plates, using a solvent mixture system of chloroform:diethyl ether (50:50). The crude mixture was then filtered, washed with a cold solution of $NaHCO_3$, and extracted with diethyl ether, to

afford the corresponding ester. 7-docosahexaenoyl quercetin: [2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl-octadec-9-enoate] was obtained in 60% yield as a brownish oil; ^{1}H NMR (400 MHz, DMSO-d_6): 7.68 (C-2″, d, J = 2.11 Hz, 1H); 7.55 (C-6″, dd, J = 2.11, 8.44 Hz, 1H); 6.89 (C-5″, d, J = 8.44 Hz, 1H); 6.40 (C-8′, s, 1H); 6.18 (C-6′, s, 1H); 5.34 (C-4,5, 7,8, 10, 11, 13, 14, 16, 17, 19, 20, m, 12H); 2.82 (C-6, 9, 12, 15, 18, m, 10H); 2.25 (C-3, m, 2H); 2.02 (C-21, m, 2H); 1.18 (C-22, t, J = 7.52 Hz, 3H). ^{13}C NMR (100.62 MHz, DMSO-d_6) 176.4, 175.7, 164.1, 161.0, 156.4, 147.8, 144.7, 136.2, 127.6, 122.4, 119.8, 115.2, 114.4, 103.4, 98.1, 93.4, 33.6, 25.1, 19.7, 13.5. ESI-MS: 613 $[M+H]^+$. Anal Calcd for $C_{37}H_{40}O_8$: C, 72.53; H, 6.58. Found: C, 72.60; H, 6.59.



Docosahexaenoic acid Quercetin 7-Docosahexaenoyl quercetin

i) docosahexaenoic acid (1 equiv.), quercetin (1 equiv.), pancreatic porcine lipase (5 eq), acetone (30 mL), 37 °C, 130 rpm, 48 h

Figure 2. Synthesis of 7-docosanoylquercetin.

Supplementary Materials: The following are available online, Figure S1: ^{1}H-NMR spectrum; Figure S2: ^{13}C-NMR spectrum.

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