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Short Note

Bio-Catalytic Synthesis of Quercetin-3-Oleate

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Abstract: Polyphenols are well-known health promoting agents, but they have some limitations due to their spontaneous oxidation. This evidence has limited their use as drugs in the last years. In this field, several chemical modifications have been proposed to overcome these restrictions; among these, the esterification seems to be the preferred. Ester derivatives could be able to reduce the bioavailability problems connected to polyphenols. On the other hand, the presence of the esterase enzymes in the body guarantees the ester hydrolysis, which in turn frees the two molecules that make it up. Lipase-catalyzed esterifications afforded several derivatives of flavonoids glycosides, in green conditions. In this short note, pancreatic porcine lipase was firstly used as a cheap bio-catalyst, to synthesize oleoyl derivatives of quercetin in aglycone form. Results demonstrated how the enzyme acyl regioselective in position C-3, with high yields and easy purification processes.

Keywords: keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article; yet reasonably common within the subject discipline.)

1. Introduction

Quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, is one of the secondary metabolites of plants, representative of the flavonoid family [1]. It has several biological activities, including antioxidant, anti-proliferative, anti-diabetic, vasodilating, anti-inflammatory and other ones. These health-promoting properties are usually limited by itsfast metabolism, which affect often the critical points of the molecule's bioactivity (i.e. OH groups) [2]. Several chemical modifications have been proposed to increase bioavailability and limit metabolism. In this context, it is well known how the glycosylation and mostly acylation reactions are used to improve flavonoids properties [3]. In the last years regioselective syntheses emerged as very important chemical processes; various researchers have proposed enzymatic reactions as very selective and high yielding methods, although often in the case of flavonoids glycosides [4]. Recently, two studies [5,6] reported the enzymatic synthesis of cinnamate and oleate esters of quercetin, demonstrating how in particular reaction's conditions it is possible get a mixture of three main products, 4'-monoester, 3',4'-diester and 7,3',4'-triester. In this short note, an eco-friendly, bio-catalytic process has been proposed to synthesize in only one step the C-3-oleate of quercetin. By using pancreatic porcine lipase (PPL), quercetin-3-oleate was obtained in very high yield, as unique product, and recycling the enzyme for five times. This study demonstrates the regioselectivity of PPL towards the C3-hydroxyl group.

2. Results

In a previous paper, a semisynthetic derivative of quercetin, 2-(2,2-diphenylbenzo[d][1,3] dioxol-5-yl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl oleate, was synthesized according to Steglich conditions and validated as a potential insulin secretagogue agent G-protein-coupled receptor 40 ligand [3]. In this context, 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl oleate, quercetin-3-oleate, was synthesized avoiding the preventive catechol protection but considering several literature observations [7], considering that Candida antartica Lipase B® (the most used acylating enzyme) was not able to produce esters of quercetin [8]. In a typical optimized experiment, 1 equivalent of quercetin, 1 equivalent of oleic acid were added of PPL and acetone. Temperature

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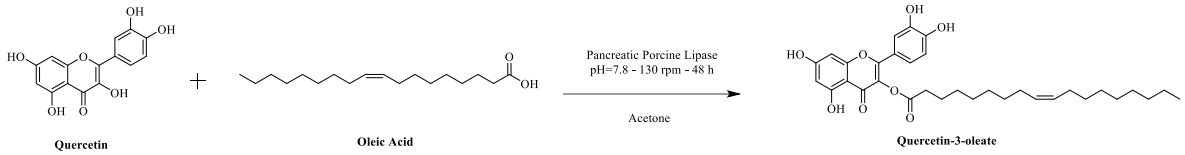
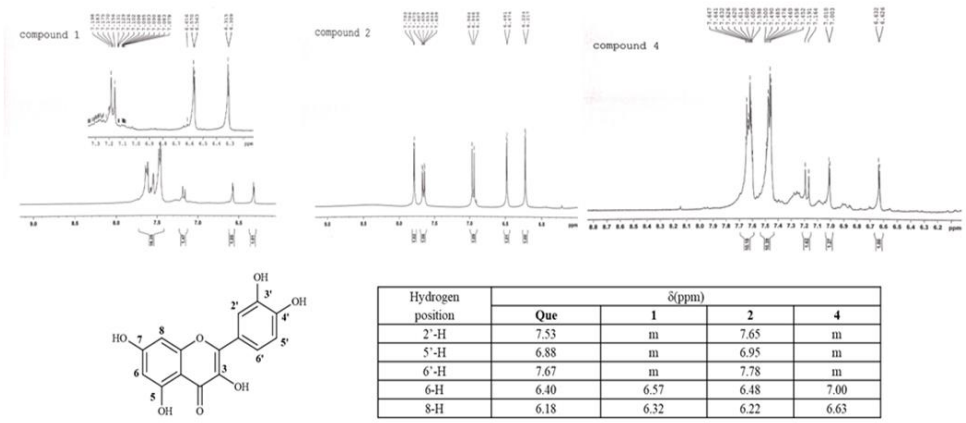
was maintained at 37°C, with an agitation rate of 130 rpm. The desired product was obtained in 88% isolated yield after 48 h incubation. The compound **2** was fully characterized by IR, ^{1}H-NMR and ^{13}C spectroscopies and elemental analysis.



Scheme 1. Representative bio-catalytic process for the preparation of quercetin-3-oleate

The catalytic triad of lipase is always: Ser-Asp/Glu-His; serine is the essential nucleophilic residue for catalysis. During the process of esterification, the first tetrahedral intermediate is formed after the attack nucleophile of the serine residue on the acid; the intermediate loses a water molecule and the acyl-enzyme complex is formed. The OH group of the intervening molecule in esterification, usually an alcohol (C3-OH in the case of quercetin) performs a nucleophilic attack on the complex just formed, giving rise to the second tetrahedral intermediate; the latter free the ester formed and the enzyme returns to its native form. The exact acylation site was determined by comparing the chemical shifts between pure quercetin and its acylated analogues just published (compounds **1** and **4** in the Figure 1). The chemicals shifts were found to be slightly shifted to downfield or upfield values [6]. For examples, substitutions at 3 and 7 hydroxyls to form quercetin 7,3-dioleate moved the chemical shifts of the neighbouring protons (6 and 8) to higher field. The presence of diphenyl methyl ketal backbone moved the chemical shifts of the 2′ and 5′ protons to lower and higher field, respectively. The mono-substitution of quercetin-3-oleate did not move the chemical shifts of 6,8 protons in a significative manner.



Hydrogen position	δ(ppm)			
	Que	1	2	4
2'-H	7.53	m	7.65	m
5'-H	6.88	m	6.95	m
6'-H	7.67	m	7.78	m
6-H	6.40	6.57	6.48	7.00
8-H	6.18	6.32	6.22	6.63

Figure 1. ^{1}H NMR (300 MHz, in Acetone-d_6) chemical shifts of the protons of the oleoyl derivatives compared to quercetin

3. Materials and Methods

The enzymes 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 Oleic acid ≥99% (GC) were purchased from Merck (Italy). Acetone was purchased from Levanchimica (Italy). The selective C-3 position of acylation was determined by NMR. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker Advance 300 spectrometer at 300 and 75 MHz,

respectively. Acetone-d_6, and DMSO-d_6 were used as solvents. IR spectrum was recorded on a PerkinElmer machine 10.4.00.

Synthetic procedure for the preparation of quercetin-3-oleate (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl-octadec-9-enoate). In a round-bottom flask, to a solution of quercetin (100 mg, 0.330 mmol) and oleic acid (93.46 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 from diethyl ether, to afford the corresponding oleate. Compound 2: Quercetin-3-oleate (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl-octadec-9-enoate) was obtained in 88% yield as a yellow resin; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.78 (d, 1H, J = 1.87 Hz), 7.64 (dd, 1H, J = 1.73 Hz, 8.50 Hz), 6.98 (d, 1H, J = 8.48 Hz), 6.50 (d, 1H, J = 1.31 Hz), 6.28 (d, 1H, J = 1.37 Hz), 5.42-5.38 (m, 2H), 2.29 (t, 2H, J = 7.3 Hz), 2.05 (m, 4H), 1.57 (m, 2H), 1.34-1.24 (m, 20H), 0.94 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.25, 174.82, 164.33, 161.16, 156.56, 148.11, 147.19, 145.47, 136.16, 130.00, 129.97, 122.41, 120.36, 115.99, 115.49, 103.42, 98.57, 93.72, 34.08, 31.70, 29.52, 29.47, 29.26, 29.11, 29.02, 28.99, 28.92, 26.99, 2.91, 22.50, 14.46, 14.29. Elemental Analysis for $C_{33}H_{42}O_8$ found C, 69.81; H, 7.60. IR: ν = 3275.59 (m), 2921.96 (s), 2852.57 (s), 1708.89 (s), 1462.81 (m), 1349.03 (s), 1301.90 (m) cm^{-1}.

Supplementary Materials: ^{1}H-NMR and ^{13}C-NMR spectra for product **2** are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

Author Contributions: G.C. and F.A. conceived and designed the experiments; G.C. performed the experiments; F.A. analyzed and confirmed the data analysis. G.C. wrote the paper; F.A. approved the final proof of the paper.

Conflicts of Interest: The authors declare no conflict of interest

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