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Preparation of regioprotected morins by lipase-catalysed transesterification☆

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Abstract

Different morin acetates have been prepared by alcoholysis of peracetate (**6**) in tetrahydrofuran with butanol, using *Candida antarctica* B or *Rhizomucor miehei* lipase as catalysts. The first enzyme recognised preferentially the acetate group located at position C-4′ on B ring, giving 3,5,7,2′-tetraacetylmorin (**7**). The second one, less selective, hydrolysed with comparable rates ester groups at position C-7 on A ring and C-4′ on B ring, furnishing **7** and 3,5,2′,4′-tetraacetylmorin (**8**) respectively. Prolonging alcoholysis time, both lipases gave a morin ester having free OH groups at C-7 and C-4′, 3,5,2′-triacetylmorin (**9**). In any case lipases did not affect ester groups located at position C-3, C-5 and C-2′. Morin derivative having free function OH in C-5, 3,2′-diacetylmorin (**11**) was prepared in high yield using as substrate for alcoholysis the partial ester 3,7,2′,4′-tetraacetylmorin (**10**) easily prepared by chemical conventional method.

Keywords: Lipase; Morin; Alcoholysis; Regioselectivity; Biocatalysis

1. Introduction

Flavonoids are proven to exhibit several biological properties, including antitumoral and anti-HIV activity [1]. Their radical scavenging action, correlated with antioxidant property, is involved in different biological processes and today well recognised [2–4]. The hydroxyl groups located on the benzopyran skeleton play a pivotal role in the chemical and biological activities of these polyphenols. Therefore, the possibility to modulate and/or selectively modify these OH functions results particularly useful in the design of new flavonoids or in the synthesis of derivatives possessing appropriate structural features for specific actions. Unfortunately the discrimination of OH functions in polyhydroxylated flavonoids is not easily accessible by conventional procedures, with the consequence of products mixtures hard and expensive to purify.

Inside a project regarding the preparation of new flavonoids of interest for application in medicine and food chemistry [5–9], we have realized selective transformations of the basic flavonoid structure by lipase-catalysed esterification/alcoholysis [10]. Now this procedure has been expanded to selectively discriminate the single OH functions located at A or B ring of morin (**1**), a flavonoid possessing potential use in medicine [11–16]. The result of this study is here reported.

2. Material and methods

2.1. Enzymes and chemicals

Lipozyme® IM (immobilised lipase from *Mucor miehei*) and Novozym® 435 (immobilised lipase B from *Candida antarctica*) are registered marks from Novo Nordisk. Morin was purchased from Sigma–Aldrich. All other solvents and chemicals were commercial and used without further purification.

2.2. Purification and structure determination

All compounds were purified by column chromatography performed on silica–gel or Lichroprep Si-Diol 40–63 μm (Merck); analytical TLC was carried out on Merck silica gel 60-F254 precoated glass plates and compounds were visualized by spraying with molybdophosporic acid.

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Structure of the products were determined by ^{1}H and ^{13}C NMR analysis, recorded in acetone-*d*6 on a Bruker Avance™ 400 instrument at 400.13 and 100.03 MHz respectively.

2.3. Chemical synthesis of morin esters

Acetic anhydride (16 mmol) was added to *t*-butylmethyl ether (*t*BME) containing morin (500 mg, 1.6 mmol) (**1**), and triethylamine (TEA, 16 mmol). The solution refluxed for 2 h until TLC analysis showed the complete conversion of the substrate and the formation of a single product. The reaction mixture was then extracted with ethyl acetate, washed with H_2O, dried over Na_2SO_4 and finally taken to dryness under vacuum to give 3,5,7,2′,4′-pentaacetylmorin (**6**) (810 mg, 96% yield).

A parallel reaction, carried out at room temperature, furnished 3,7,2′,4′-tetraacetylmorin (**10**) (740 mg, 95% yield).

2.3.1. 3,5,7,2′,4′-Pentaacetylmorin (*6*)

^{1}H NMR δ: 2.15 (s, Ac); 2.20 (s, Ac); 2.29 (s, Ac); 2.30 (s, Ac); 2.35 (s, Ac); 7.03 (d, $J = 2.0$ Hz, H-6); 7.22 (d, $J = 2.1$ Hz, H-3′); 7.26 (dd, $J = 8.5$, 2.1 Hz, H-5′); 7.37 (d, $J = 2.0$ Hz, H-8); 7.76 (d, $J = 8.5$ Hz, H-6′). ^{13}C NMR δ: 20.10; 20.80; 20.84; 20.93; 21.28; 110.14; 115.24; 115.60; 118.52; 120.58; 120.95; 131.75; 150.13; 151.37; 153.85; 154.37; 155.60; 158.08; 167.97; 168.76; 169.12; 169.23; 169.29; 170.00; 170.85.

2.3.2. 3,7,2′,4′-Tetraacetylmorin (*10*)

^{1}H NMR δ: 2.18 (s, Ac); 2.20 (s, Ac); 2.30 (s, Ac); 2.31(s, Ac); 6.68 (d, $J = 2.0$ Hz, H-6); 6.92 (d, $J = 2.0$ Hz, H-8); 7.22 (d, $J = 2.2$ Hz, H-3′); 7.29 (dd, $J = 8.5$, 2.2 Hz, H-5′); 7.78 (d, $J = 8.5$ Hz, H-6′). ^{13}C NMR δ: 18.89; 19.68; 19.84; 20.15; 101.23; 105.21; 108.34; 117.46; 119.50; 119.52; 130.68; 149.16; 151.40; 153.54; 155.07; 156.26; 156.74; 161.32; 166.93; 167.68; 167.98; 168.07; 175.89.

2.4. Enzymatic reactions

In a general procedure lipase (100 mg) was added to a solution of the chosen flavonoid (100 mg) in THF (10 mL) containing *n*-butanol (2 equiv.). The suspension was shaken (300 rpm) at 45° and the reaction monitored by TLC. At regular times aliquots of the reaction mixture were withdrawn and investigated by ^{1}H NMR analysis to determine the conversion until desired value. The reaction was then quenched, filtering off the catalyst and the filtrate evaporated to dryness in vacuo. The residue was purified by column chromatography on Lichroprep Si-Diol 40–63 μm (AcOEt-*n*-hexane gradient) to furnish the alcoholysis products.

2.4.1. 3,5,7,2′-Tetraacetylmorin (*7*)

^{1}H NMR δ: 2.32 (s, 2× Ac); 2.17 (s, Ac); 2.13 (s, Ac); 6.79 (d, $J = 2.3$ Hz, H-3′); 6.92 (dd, $J = 8.5$, 2.3 Hz, H-5′); 7.00 (d, $J = 2.1$ Hz, H-6); 7.35 (d, $J = 2.1$ Hz, H-8); 7.56 (d, $J = 8.5$ Hz, H-6′). ^{13}C NMR δ: 19.00; 19.81; 20.13; 108.91; 109.10; 110.55; 113.11; 113.58; 113.94; 130.96; 150.94; 151.36; 155.02; 155.48; 155.64; 158.01; 161.72; 168.05; 168.81; 169.15; 169.31; 170.07.

2.4.2. 3,5,2′-Triacetylmorin (*9*)

^{1}H NMR δ: 2.29 (s, Ac); 2.18 (s, Ac); 2.11 (s, Ac); 6.65 (d, $J = 2.0$ Hz, H-6); 6.78 (d, $J = 2.2$ Hz, H-3′); 6.82 (d, $J = 2.0$ Hz, H-8); 6.91 (dd, $J = 8.5$, 2.2 Hz, H-5′); 7.51 (d, $J = 8.5$ Hz, H-6′). ^{13}C NMR δ: 19.84; 19.90; 2013; 100.43; 108.83; 109.98; 110.51; 113.00; 113.9; 130.86; 149.79; 151.05; 152.95; 158.18; 160.33; 162.18; 167.03; 168.12; 168.17; 168.86.

2.4.3. 3,5,2′,4′- Tetraacetylmorin (*8*)

^{1}H NMR δ: 2.30 (s, Ac); 2.29 (s, Ac); 2.20 (s, Ac); 2.11 (s, Ac); 6.67 (d, $J = 2.3$ Hz, H-6); δ 6.84 (d, $J = 2.3$ Hz, H-8); 7.19 (d, $J = 2.2$ Hz, H-3′); 7.26 (dd, $J = 8.5$, 2.2 Hz, H-5′); 7.74 (d, $J = 8.5$ Hz, H-6′). ^{13}C NMR δ: 101.61; 110.21; 110.90; 118.54; 120.54; 121.33; 131.70; 135.35; 138.13; 150.11; 152.15; 152.81; 154.20; 159.38; 163.86; 168.12; 169.15; 169.23, 169.33; 169.81.

2.4.4. 3,2′-Diacetylmorin (*11*)

^{1}H NMR δ: 2.18 (s, Ac); 2.15 (s, Ac); 6.31 (d, $J = 1.9.1$ Hz, H-6); 6.43 (d, $J = 1.9$ Hz, H-8); 6.79 (d, $J = 2.2$ Hz, H-3′); 6.92 (dd, $J = 8.5$, 2.2 Hz, H-5′); 7.50 (d, $J = 8.5$ Hz, H-6′). ^{13}C NMR δ: 18.97; 19.85; 93.78; 98.99; 104.35; 110.62; 113.13; 113.98; 130.97; 149.89; 155.02; 157.45; 160.69; 162.11; 164.61; 167.16; 168.16; 168.26; 175.31.

3. Results and discussion

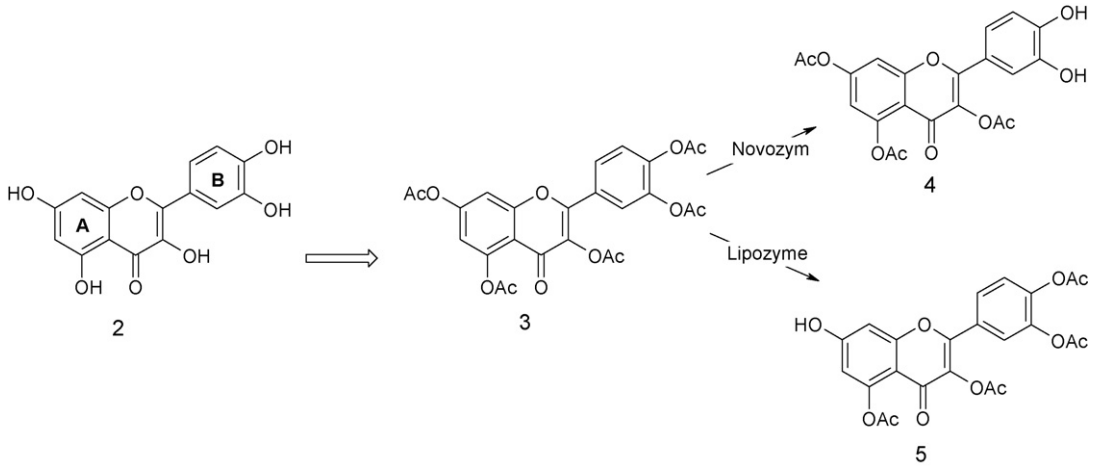
In previous investigations aimed to prepare lipophilic esters of quercetin **2** selectively protected to A or B ring, we evidenced an opposite regiopreference for *C. antarctica* B (as Novozym 435) and *Rhizomucor miehei* (Lipozyme IM) lipases [10]. Thus alcoholysis of peracetate (**3**) in the presence of Novozym 435 furnished triester **4** as a favoured product bearing the ring B free.

Table 1
Regioalcoholysis of morin acetates catalysed by *Candida antarctica* B (Novozym 435) and *Rhizomucor miehei* (Lipozyme IM) lipases in THF

Substrate	Lipase	React time (h)	Conversion (%)	Ring recognised	Product (%)
6	Novozym 435	12	45	B	**7** (40)
		48	100	A+B	**9** (90)
6	Lipozyme	8	40	A	**8** (20)
				B	**7** (20)
		48	100	A+B	**9** (90)
10	Novozym 435	2	100	A+B	**11** (95)

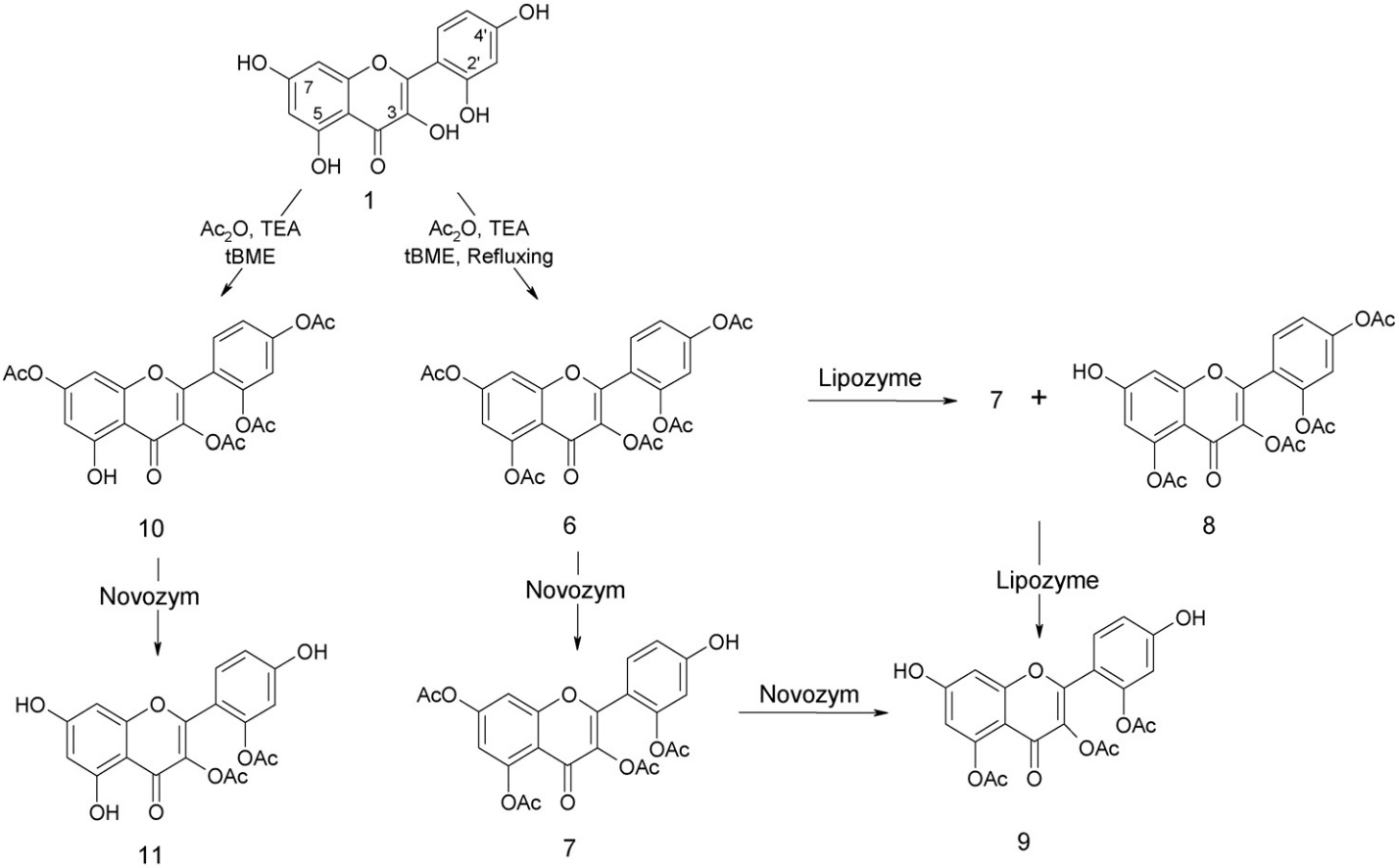
Conversely, when Lipozyme was used, alcoholysis involved the acetyl group located at C-7 position and the tetraester **5** was isolated.

Alcoholysis of **6** in presence of *R. miehei* lipase was less selective: Lipozyme recognises both rings, giving the hydrolysis of acetate in C-7 or in C-4′ with comparable rates. At 40% of conversion only the tetraacetates (**7**) and (**8**) were present in solution; by prolonging reaction time, further alcoholysis of these derivatives converges toward triester **9** isolated in 90% yield. In the above experiments no alcoholysis at phenolic positions 5 and 2′ was observed, and the acetate group in allylic position C-3 lies untouched. Considering this trend, both employed lipases turned useless in preparation of morin derivatives bearing OH free in C-5. To overcome this hitch an alternative can be the use of ester



2 3 4 5

With this result in mind, we have applied these reactions to prepare derivatives of morin (**1**), structurally similar to quercetin. Morin pentaacetate (**6**), synthesized by conventional acetylation under refluxing conditions, was subjected to alcoholysis with *n*-butanol in tetrahydrofuran in the presence of Novozym 435 and the preferential deprotection of the acetate function at C-4′ was observed, furnishing **7** in moderate yield. Advantageously the acetate in C-2′ does not suffer any hydrolysis, showing a different behaviour of morin respect to the analogous quercetin. However, ester **7** suffers alcoholysis at the C-7 position and triester **9** is the final product recovered in solution at long reaction times.



1 10 6 8 11 7 9

10 as substrate, easily prepared by conventional chemical route operating at controlled temperature (at low room temperatures the presence of a strong hydrogen bonding between 5-OH and the carbonyl function prevents its acetylation). Thus when **10** was subjected to alcoholysis in the presence of Novozym 435, surprisingly the reaction occurred very fast to give unexpected cleavage of the ester group in C-7 together with the predictable one in C-4′, furnishing quantitatively diester **11** in 2 h. Table 1 summarises the conversions yields and the morin esters obtained for all reactions performed.

4. Conclusions

In conclusion we have developed a simple and convenient biocatalytic procedure valuable for the discrimination of phenolic functions on A or B ring in morin. The biocatalysed alcoholysis of morin peracetate has the result to furnish partial esters not synthesized by conventional methods until now; it can be noticed that the enzymatic reactions of morin esters are fully controlled by steric effects, infact only the least hindered ones are recognised by the employed lipases. The new products, bearing free specific OH groups on the morin framework are useful starting material for further synthetic modification. Studies are at work in our laboratory to transform them in lipophilic morin of potential application as radical scavengers.

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