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ScienceDirect
Chinese Chemical Letters 21 (2010) 59–62
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

Regioselective enzymatic acylation of troxerutin in nonaqueous medium

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Received 8 June 2008

Abstract

A series of monosubstituted troxerutin esters have been synthesized by enzyme-catalyzed regioselective acylation of troxerutin in nonaqueous medium. Using divinyl dicarboxylates (CH_2═CH–OOC–$(CH_2)_n$–COO–CH═CH_2, n = 2, 3, 4, 7, 8, 11) featuring different chain length as acyl donors and alkaline protease from *Bacillus subtilis* as catalyst, troxerutin was regioselective acylated at B' ethoxyl group. The results indicated that the regioselectivity of the enzyme-catalyzed acylation was not affected by the chain length of the acyl donor.

Keywords: Troxerutin; Acylation; Enzymatic synthesis; Regioselectivity

Flavonoids are widely used in food, cosmetics, medicines and various commodity preparations [1] due to their antioxidant, antimicrobial, anti-inflammatory, anti-tumor and anti-angiogenic activities [2,3], while their application is still limited by their low stability and solubility in lipophilic or hydrophilic media [4]. In order to improve their stability or modify the solubility, many groups have studied the derivatization of flavonoids by chemical, enzymatic and chemoenzymatic methods. Biocatalysts with high specificity and selectivity have gained recognition as favorable alternatives to catalyze the acylation of flavonoids [5–7]. It has been reported that regioselective acylation of flavonoids enhanced their antioxidant and antimicrobial activities [8].

Enzymatic acylation of flavonoids provides a relatively efficient synthetic approach to the availability of a large range of flavonoids derivatives, which would facilitate the investigation of their biological properties. In the paper, we investigated the regioselective enzymatic acylation of troxerutin and synthesized a series of monosubstituted troxerutin esters. The obtained troxerutin derivative containing a vinyl group may be further utilized as precursor of functional material or monomer of polymeric drug.

1. Experimental

Alkaline protease from *Bacillus subtilis* was from Wuxi Enzyme Co. Ltd. (4500 U/mg, P. R. China). Troxerutin was provided by Sichuan Yabao Guangtai Pharmaceutical Co. Ltd. (P. R. China). All other chemicals were of the highest purity commercially available. Divinyl carboxylates were prepared and purified as described by the patent [9].

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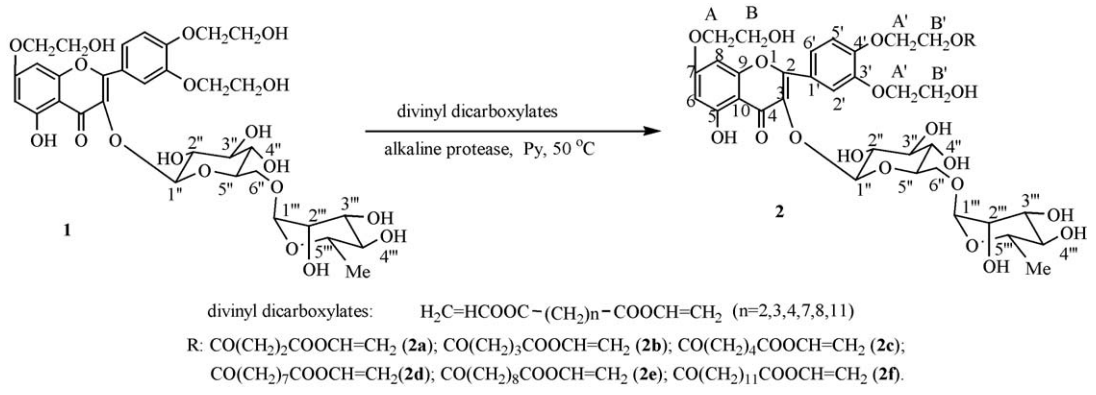
1001-8417/$ – see front matter
doi:10.1016/j.cclet.2009.08.017



Scheme 1. Enzymatic synthesis of vinyl troxerutin esters.

The process of the reaction was monitored by TLC. Infrared spectra were measured with a Nicolet Nexus FTIR 670. The acylation position of the product was established by ^{1}H NMR and ^{13}C NMR (Bruker Advance DMX 500). Mass spectra were obtained on a Bruker Esquire-LC instrument (methanol; negative mode).

To initiate the reaction, 2.5 g alkaline protease from *B. subtilis* was added to the mixture of troxerutin (2.8 g, 0.00377 mol) and divinyl dicarboxylate (0.015 mol) in pyridine (100 mL). The suspensions were shaken at 250 rpm for 5 days at 50 °C. The reaction was followed by TLC (eluent, ethyl acetate/methanol/H_2O, 15:3.6:1, v/v) and terminated by filtering off the enzymes. The filtrates were concentrated under reduced pressure and the products were obtained by column chromatography purification. Enzymatic synthesis of troxerutin esters **2a**–**2f** was shown in Scheme 1.

2. Results and discussion

Transesterification of troexrutin with divinyl dicarboxylates in the presence of alkaline protease from *B. subtilis*, afforded monosubstituted troxerutin derivatives **2** (Scheme 1). The products obtained were characterized by ESI-MS, FT-IR, ^{1}H NMR and ^{13}C NMR.

In the ^{13}C NMR spectrum of **2c** (Table 1), the signal of C-A′ upfielded from 70.78 to 67.41 ppm, and the signal of C-B′ downfielded from 60.03 to 62.98 ppm, while the chemical shifts for C-A and C-B did not change obviously, indicating that the structure of the esterification product should be **2** [10]. ^{13}C NMR spectra analysis of **2a**–**2f** indicated that the transesterifications all selectively occurred at the B′ primary hydroxyl position, not B primary hydroxyl

Table 1
^{13}C NMR data of troxerutin and vinyl derivatives (DMSO-d_6, δ).

Carbon number	Troxerutin	2a	2b	2c	2d	2e	2f
C-4	177.97	177.95	177.94	178.11	177.92	177.96	177.93
C-7	165.15	165.13	165.11	165.29	165.11	165.14	165.11
C-9	161.34	161.30	161.27	161.46	161.27	161.31	161.27
C-5	157.05	156.94	156.90	157.09	156.89	156.93	156.89
C-2	156.95	156.88	156.10	157.04	151.45	156.88	151.47
C-4′	151.37	151.54	150.73	151.66	148.06	151.53	150.78
C-3′	148.02	148.15	148.06	148.29	147.49	148.14	148.07
C-3	134.16	134.24	134.19	134.40	134.21	134.26	134.22
C-1′	123.05	123.81	123.37	123.85	122.80	123.72	123.35
C-6′	122.88	123.04	122.99	123.01	122.70	123.06	123.00
C-5′	114.90	113.89	114.90	115.66	115.34	115.55	116.18
C-2′	113.29	113.58	113.50	113.72	115.13	113.86	115.44
C-10	105.55	105.53	105.52	105.69	105.48	105.54	105.48
C-1″	101.78	101.81	101.70	101.91	101.85	101.88	101.86
C-1‴	101.41	101.36	101.38	101.46	101.37	101.37	101.37
C-6	98.87	98.85	98.85	99.01	98.84	98.86	98.84
C-8	93.39	93.36	93.33	93.51	93.32	93.36	93.32

Table 1 (*Continued*)

Carbon number	Troxerutin	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**
C-3″	76.86	76.84	76.79	76.99	76.79	76.85	76.80
C-5″	76.45	76.40	76.36	76.57	76.38	76.42	76.38
C-2″	74.65	74.61	74.60	74.76	74.59	74.61	74.59
C-4‴	72.23	72.21	72.15	72.35	72.15	72.20	72.17
C-3‴	71.08	71.13	71.03	71.29	71.05	71.13	71.04
C-2‴	71.08	71.07	71.03	71.11	71.04	71.07	71.03
C-4″	70.99	70.86	70.94	71.00	70.94	70.96	70.94
C-A′	70.82	70.78	70.77	70.94	70.77	70.80	70.77
C-A′	70.82	67.24	67.40	67.41	67.42	67.28	67.45
C-A	70.67	70.60	70.58	70.78	70.59	70.63	70.59
C-5‴	68.76	68.70	68.40	68.87	68.70	68.72	68.71
C-6″	67.62	67.57	67.16	67.60	67.22	67.59	67.60
C-B′	60.03	60.00	59.96	60.12	59.98	60.01	59.97
C-B′	60.03	63.24	62.89	62.98	62.76	62.77	62.89
C-B	59.79	59.75	59.72	59.91	59.72	59.75	59.72
C-6‴	18.18	18.13	18.15	18.30	18.14	18.15	18.14
C=O		173.34	172.88	173.34	173.40	173.40	173.41
		170.01	170.49	170.87	170.86	170.87	170.87
$OCH{=}CH_2$		141.61	141.64	141.83	141.64	141.70	141.67
$OCH{=}CH_2$		98.67	98.57	98.65	98.42	98.43	98.42
$(CH_2)_n$		28.79	33.36	33.61	33.80	33.88	33.85
			32.77	33.28	33.42	28.97	33.46
			32.50	24.30	28.75	24.81	29.37
					28.61		29.32
					24.77		29.30
					24.42		29.18
							29.09
							28.91
							28.78
							24.86
							22.48

position. There are three primary hydroxyl groups in the troxerutin derivative and the DS (Degree of Substitution) of the products was determined by ^{1}H NMR and MS. In the ^{1}H NMR spectrum of **2c**, the ratio of proton integral for (CH_2)n groups and troxerutin showed the product to be monosubstituted ester. The ESI-MS analyses were in accordance with the ^{1}H NMR results. Similar results were observed in **2a**–**2f**.

Enzyme is an important factor for selectivity and activity. Six other commercially available enzymes (CCL, Lipozyme, PPL, Novozym 435, HPL and Alkaline protease from *Bacillus licheniformis*) were chosen for the transesterification. The results showed that CCL, Lipozyme, PPL and HPL did not show any reactivity in pyridine even after 7 days, while alkaline protease from *Bacillus licheniformis* showed similarly catalytic activity as alkaline protease from *Bacillus subtilisin*.

The obtained vinyl troxerutin esters have high application potential as precursor of functional material in pharmaceutical and material chemistry. Studies on the reaction of vinyl troxerutin and metformin are being in progress.

Acknowledgments

We thank the Science and Technology hall of Henan Province (No. 072300420070; No. 82102330014), China, for financially supported to this work.

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