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Note

Enzyme catalyzed acylation of 7-hydroxy-4-methyl-2*H*-chromene-2-one using microwave

Mazaahir Kidwai*, Poonam Mothsra & Roona Poddar

Green Chemistry Research Laboratory, Department of Chemistry, University of Delhi, Delhi 110 007, India

E-mail: kidwai.chemistry@gmail.com

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Acylation of pharmacologically important 7-hydroxy-4-methyl-2*H*-chromene-2-one has been investigated in the presence of immobilized lipase under the influence of microwave. Commercially available lipase (Novozyme 435) under microwave leads to enhancement in rate of reaction in comparison with conventional heating in immobilized lipase catalyzed acylation with various acids. Different microwave assisted technologies were studied and compared. This paper investigates the synergism between enzyme catalysis and microwaves on acylation of 7-hydroxy-4-methyl-2*H*-chromene-2-one, with different acids using lipase Novozyme 435.

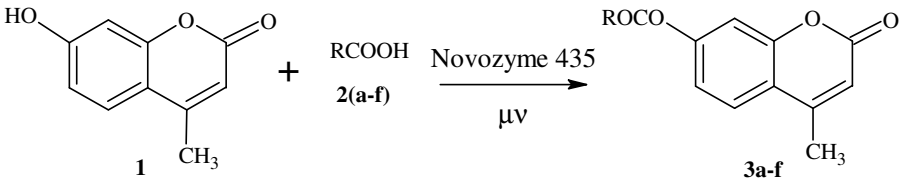
Keywords: Thermal microwave irradiation, immobilized lipase catalysis, 7-hydroxy-4-methyl-2*H*-chromene-2-one, synergism

Since last decade there has been a stress to work in the area of Green Chemistry[1]. To achieve such goal, elimination of hazardous chemicals which minimizes waste generation and benign sustainable courses are required. Much work in this direction for synthetic methodologies has been adopted[1] for chemical processes[2]. Utilization of non-toxic chemicals, environmentally benign solvents, and renewable materials are some of the key issue that merit important consideration in green synthetic strategies[3].

There has been growing interest in the use of enzymes in the production of fine chemicals, pharmaceuticals, fuels and numerous other manufactured goods[4]. Enzymes are superior catalysts able to accept a wide array of complex molecules as substrates and catalyze reactions at mild conditions. Further more biocatalysis rather than conventional chemistry are more compatible with 'Green chemistry', 'environmentally benign production processes and sustainable development[5]. Lipases are the most widely used enzymes because they are cheap, easily available, cofactor free and have broader substrate specificity[6].

One of novel approaches towards clean and green chemistry is the application of microwaves, which is relatively a convenient, safe and rapid methodology as reported by our lab[7]. Their application in enzyme catalyzed reaction is relatively limited. First studies carried out in aqueous solution with various enzymes did not demonstrate any effect of microwave field on enzymatic activity and stability[8]. More recently, still in an aqueous medium it was reported that inactivation of a pectin methylesterase was faster in microwave heating mode, suggesting the presence of non-thermal effects under microwave irradiation[9]. Similar results were obtained with thermophilic enzymes, on which microwave irradiation has induced protein structural rearrangements not related to temperature[10]. On the other hand, synergisms of microwave and immobilized enzyme catalysis in non-aqueous media have been reported[11]. Thus, it was thought to study some transformation of pharmacologically important moieties simultaneously under microwave and enzyme catalysis. Thus, 7-hydroxy-4-methyl-2*H*-chromene-2-one are members of the class of compounds called benzopyrones and diverse pharmacological properties[12] with natural[13] and synthetic origin[14]. The activity of chromenones derivatives as anti coagulants and antithrombotics is well known[15]. 4-Hydroxy-3-substituted-2*H*-chromen-2-one[16] was reported to possess HIV inhibitory potency. It is therefore of utmost importance to design new coumarin derivatives or alteration of chemical structure of these molecules by simple, effective and environmentally benign. Consequently, acylation of 7-hydroxy-4-methyl-2*H*-chromene-2-one was tried.

Several routes are available for acylation in literature[17] but most of these methods have problems such as difficulty in handling the reagents, using toxic and hazardous solvents and are limited for wide applications. Lipase (Novozyme 435) has been used to acylate a variety of substrates, including natural products, such as oligosaccharides[18], diterpenes[19], flavonoids[20] and triterpenoid saponins[21]. This paper investigates the synergism between enzyme catalysis and microwaves on acylation of 7-hydroxy-4-methyl-2*H*-chromene-2-one, with different acids using lipase Novozyme 435 **(Scheme I)**.



Scheme I

Results and Discussion

Lipases are the most widely used enzymes because they are cheap, easily available, cofactor free and have broader substrate specificity. Lipase (triacyl glycerol hydrolases EC 3.1.1.3) catalyzes hydrolysis, esterification, transesterification, thioesterification, amidation, epoxidation etc. So, there are wide ranges of literature available for lipase catalyzed reactions for acylation of aliphatic and aromatic hydroxyl group[22].

Stability and reusability of enzyme under MW

The catalyst resuability studies were carried out to determine the stability of the enzyme under microwave irradiation. After each experiment, the enzyme was filtered and washed three to four times and dried in air at RT. There was marginal decrease in activity after three reuses, which might be due to loss of enzyme during handling. Thus it can be concluded that the enzyme does not get deactivated or denatured due to microwave irradiation (**Figure 1**).

Conventional heating vs. microwave irradiation

It was found that the overall conversion as well as rate of reaction was higher under microwave irradiation *vis-a-vis* the conventional heating (**Table I**). This control experiments in the absence of Novozyme 435 did not show any conversion. Further, only microwave irradiation without the enzyme also did not initiate the reaction. Thus, there is a definite synergism between enzyme catalysis and microwave irradiation. Advantage of microwaves in terms of reactivity lies in the fact that the associated rate enhancements may allow the reaction to go to completion wherein it is barely attainable under classical heating.

Microwave assisted solution phase reaction *vs* solid supported reaction

Higher yields with shorter reaction time were obtained in case of microwave-assisted dry media reaction (solid supported reaction) as compared to microwave assisted solution phase reaction (**Table I**). The reason can be attributed to the fact that microwave irradiation coupled with dry media facilitate the evaporation of byproducts. Thus equilibrium shift in forward direction by evaporation of secondary reaction products such as water or methanol which strongly interact with the electromagnetic radiation. Control of water activity also takes place in dry media due to evaporation of water molecule which prevent other side reactions such as hydrolysis of product.

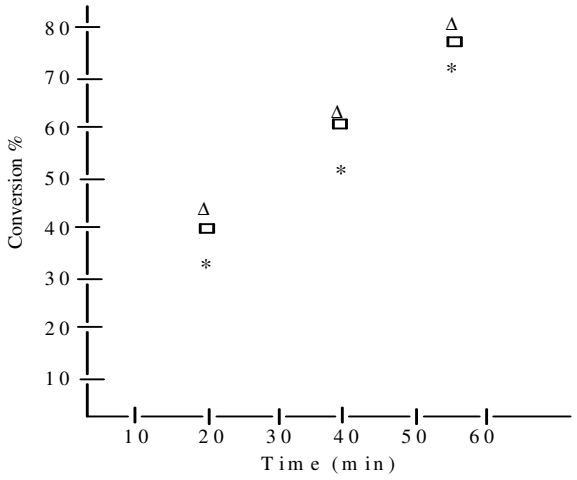


Figure 1: Screening of stability of catalyst for the acylation of 7-hydroxy-4-methyl-2*H*-chromen-2-one with acetic acid

Experimental Section

Enzyme and chemicals

The enzyme Novozyme 435 was procured as gift sample from Novo Nordisk, Denmark. Novozyme 435 is *Condida antarctica* lipase immobilised on a macroporus polyacrylic resin. All chemicals were AR grade, procured from firms of reputo and used without any further purification: 7-Hydroxy-4-methyl-2*H*-chromene-2-one (Lancaster); isopropyl ether, various acids (all from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India).

^{1}H NMR were recorded on a Bruker Avance Spectrospin 300 (300 MHz) using TMS as internal standard. IR spectra were obtained on a Perkin-Elmer FTIR-1710 spectrophotometer using Nujol film. Mass

Table I — Reaction time and yield for the laccase catalyzed acylation of 7-hydroxy-4-methyl-2*H*-chromene-2-one **3a-g**

Compd.	R	Conventional method Time (hr)/Yield (%)	MW solution phase Time (min.)/Yield (%)	MW dry media Time (min.)/Yield (%)
3a	CH_3	5/76	50/83	25/91
3b	C_6H_5	7/68	55/87	30/93
3c	4-$CH_3C_6H_4$	7/69	54/81	20/87
3d	Furyl	6/65	55/89	25/94
3e	$CH_3(CH_2)_9$–	5/72	55/90	30/97
3f	$CH_3(CH_2)_{11}$–	6/73	50/87	25/94

spectra were recorded on a JEOL JMS-DX 303. The temperature of the reaction-mixture was measured through a infrared thermometer (AZ, Mini Gun type, model 8868). The purity of compounds was checked by TLC on aluminium plates coated with silica gel (Merck). A Kenstar microwave oven at 2450 MHz was used for MWI.

Experimental conventional procedure

Substrates, 7-Hydroxy-4-methyl-2*H*-chromene-2-one and acid in isopropyl ether were placed in equimolar amounts in a 50 mL round bottom flask and heated in an oil-bath (conventional thermal heating) at 50-55°C. Nonozyme 435 was added rapidly to the substrate solution and the mixture was maintained for 6-8 hr. Periodically reaction medium was drawn and the formation of product was checked by TLC. After formation of product as checked by TLC, reaction-mixture was filtered to remove immobilized enzyme, diluted with water, precipitate formed was filtered, dried and purified further by column chromatography.

Experimental procedure of microwave-assisted solution phase reaction

7-Hydroxy-4-methyl-2*H*-chromene-2-one and acid were taken in an erlenmeyer flask with isopropyl ether as solvent. The following reaction-mixture was then irradiated under microwave for sufficient interval of time (indicated in **Table I**) and formation of reaction-mixture was periodically monitored at 30 s irradiation. Indicated by TLC, the reaction was then worked up in the similar way as discussed above in conventional method.

Experimental procedure of microwave-assisted dry media reaction

7-Hydroxy-4-methyl-2*H*-chromene-2-one and acid were dissolved in minimum amount of isopropy ether (saturated solution) and impregnated on 500 mg immobilized enzyme by subsequent evaporation of these under vaccum. Loaded accural support was taken in a beaker with dummy load and the reaction was performed in an open vessel. The dry media was irradiated under MW for sufficient interval of time (**Table I**) and the formation of the product was checked by TLC. Product was eluted from support when treated/washed with iso-propyl ether (15-20 mL), followed by dilution, filteration and purification by column chromatography.

Spectroscopic data of products 3a-f

4-Methyl-2-oxo-2H-chromene-7yl acetate 3a: m.p. 142-44°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (3H, s, $COCH_3$), 2.43 (3H, s, CH_3-4), 6.27 (s, 1H, H-3), 6.99 (1H, dd, *J* = 1.97, 8.6 Hz, H-6), 7.10 (1H, d, *J* = 1.97 Hz, H-8), 7.58 (1H, d, *J* = 8.6 Hz, H-5); IR (nujol, cm^{-1}): 2923, 2371, 1710 (C=O), 1607.

4-Methyl-2-oxo-2H-chromene-7-yl benzoate 3b: m.p. 154-56°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (3H, s, CH_3-4), 6.30 (s, 1H, H-3), 7.18 (1H, d, *J* = 2.6, 8.6 Hz, H-6), 7.24 (1H, d, *J* = 2.6 Hz, H-8), 7.50-7.67 (m, 4H, Ar-H), 8.17 (m, 2H, Ar-H, H-2',6'); IR (nujol cm^{-1}): 2925, 2367, 1712, 1610.

4-Methyl-2-oxo-chromene-7-yl-(4-methylbenzoate) 3c: m.p. 156-58°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, 4'-CH_3), 2.40 (s, 3H, CH_3-4), 6.25 (s, 1H, H-3), 7.14 (1H, dd, *J* = 2.1, 8.2 Hz, H-6), 7.16 (1H, d, *J* = 2.4 Hz, H-8), 7.42-7.61 (m, 3H, Ar-H, H-5, 3', 5'), 8.04 (m, 2H, Ar-H, H-2', 6'); IR (nujol cm^{-1}): 2923, 2369, 1711, 1608.

4-Methyl-2-oxo-chromene-7-yl-furyl 3d: m.p. 174-76°C; ^{1}H NMR (300 MHz, $CDCl_3$, DMSO): δ 2.41 (s, 3H, CH_3-4), 6.23 (s, 1H, H-3), 6.61 (1H, dd, *J* = 7.1, 6.8 Hz, H-3'), 7.08 (1H, dd, *J* = 2.4, 8.2 Hz, H-6), 7.11 (1H, d, *J* = 2.2 Hz, H-8), 7.25 (1H, d, *J* = 6.8 Hz, H-2'), 7.65 (m, 2H, H-5,4'); IR (nujol cm^{-1}): 2923, 2364, 1715, 1607.

4-Methyl-2-oxo-chromene-7-yl-caprylate 3e: m.p. 158-60°C; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 0.91 (t, 3H, H-10'), 1.28 (12H, H-4' to H-9'), 1.58 (m, 2H, H-3'), 2.38 (t, 2H, H-2'), 2.42 (s, 3H, CH_3-4), 6.28 (s, 1H, H-3), 7.06 (1H, dd, *J* = 2.0, 8.2 Hz, H-6), 7.10 (1H, d, *J* = 2.2 Hz, H-8), 7.51 (1H, d, *J* = 8.8 Hz, H-5); IR (nujol, cm^{-1}): 1732 (C=O).

4-Methyl-2-oxo-chromene-7-yl-laurate 3f: m.p. 162-64°C; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 0.90 (t, 3H, H-12'), 1.29 (16H, H-4' to H-11'), 1.56 (m, 2H, H-3'), 2.6 (t, 2H, H-2'), 2.41 (s, 3H, CH_3-4), 6.28 (s, 1H, H-3), 7.10 (1H, dd, *J* = 2.2, 8.2 Hz, H-6), 7.12 (1H, d, *J* = 2.2 Hz, H-8), 7.50 (1H, d, *J* = 8.6 Hz, H-5); IR (nujol, cm^{-1}): 1728 (C=O).

Conclusion

The reactions of 7-hydroxy-4-methyl-2*H*-chromene-2-one with different acids were studied under microwave assisted dry media and solution phase as well as conventional heating. Acylation of 7-hydroxy-4-methyl-2*H*-chromene-2-one was chosen for transformation considering their pharmacological importance. All the transformation performed under microwave irradiation was found to be faster than those under conventional conditions. The advantage of MW irradiation when compared to classical heating is evident. MW assisted solventless dry media reactions were even better than MW assisted solution phase reaction in terms of reaction rate and yield. Reusability of enzyme concluded that the enzyme does not get deactivated or denatured due to MW irradiation. The environmentally benign nature of the reaction is high since we take the added advantage of two ecofriendly technologies, which are complementary in nature.

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