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Note

An efficient and regiospecific biocatalytic esterification of some organic acids using beef pancreas lipase (Bpl)

H N Roy* M S Sarkar & A M Paul

Department of Chemistry, University of Rajshahi, Rajshahi 6205, Bangladesh

E-mail: hnroy01@yahoo.com

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Treatment of some aromatic and three dicarboxylic acids with methanol in the presence of beef pancreas lipase (Bpl) results in excellent yields of corresponding esters. The esterification is regiospecific to aromatic ring connected carboxylic functionalities.

Keywords: Biocatalytic esterification, dicarboxylic acids, beef pancreas lipase (Bpl)

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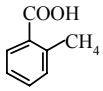
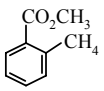
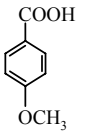
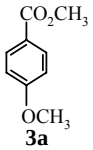
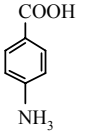
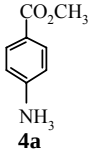
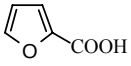
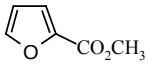
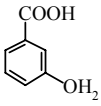
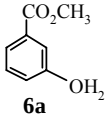
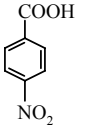
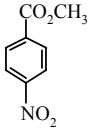
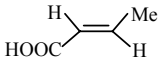
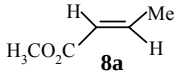
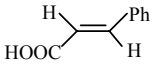
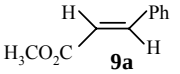
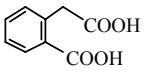
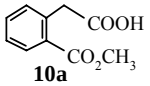
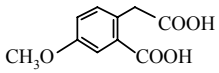
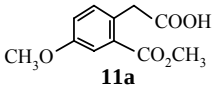
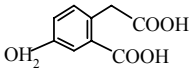
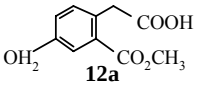
Esterification using mineral acids[1] are of limited use due to the reversibility of reactions, length of reaction time and above all the undesirable side reactions with other parts of the reacting fatty acids. Preparation of higher esters by purely chemical means requires higher energy consumption of acid catalysts and result in the formation of some toxic and coloured byproducts[2]. Esterification of crotonic and acrylic acid by H_2SO_4 often leads to polymeric products[3]. Today, *p*-toluenesulfonic (Tosic) acid is widely used for esterification in lieu of mineral acids[4]. Hosangadi *et al.*[5] have focussed on an important method for the synthesis of different esters from various acids by $SOCl_2$ treatment. Undoubtedly, this is a general method and can readily be performed under normal conditions. But, during the esterification of crotonic, cinnamic and acrylic acid by $SOCl_2$ we obtained some polymeric products in addition to a few percentage of chloro-esters[6] and in one of our experiments regiospecific esterification to one $-CO_2H$ functionality of a dioic acid by $SOCl_2$ failed even at lower temperature.

Recently, many lipases like *Candida rugosa* lipase[7] (CrI), Porcine pancreas lipase[8] (Ppl) and *Mucor javanicus* lipase[9] (MjI) have been widely used for the regiospecific esterification of some fatty acids. We have made an attempt to isolate a biocatalyst from 'Beef Pancreas' which has been successfully employed for esterification to some mono and dioic acids. In bulk methanol dioic acids with aliquot amount (≈5 mg) of Bpl lead to the formation of three monoesters.

Results and Discussion

For the test case, simple organic acids with different substituents were taken in bulk methanol at ambient temperature and aliquot amount of Bpl was added to it. It was found esterification cleanly occurred on to carboxylic groups over a period of 8~12 hr. For the extension and generalisation of our study, it has been selected 12 different acids to prove the efficacy towards Bpl esterification. Interestingly, we obtained excellent yields in all the cases except some complications arised during the esterification of amino and unsaturated acids. Unsaturated acids underwent esterification by long time exposure in methanol and only 50% yield was obtained. But in case of amino acids, Bpl did not bring about any changes. Double bonds, phenolic -OH, -Cl, $-NH_2$ and aliphatic carboxylic acid functionality were unaffected during the esterification. In addition, regiospecificity was proved by the esterification of three different dicarboxylic acids having one $-CO_2H$ functionality connected to benzene ring. Benzoic acid esterified readily (4 hr) in MeOH but for homophthalic acid (entry 10), only aromatic ring connected $-CO_2H$ group esterified quickly. Long time exposure did not bring any esterification to aliphatic part of the $-CO_2H$ group for entry 10. For further verification, homophthalic acid was esterified[5] by $SOCl_2$/MeOH. Here, two acid functionalities were esterified cleanly within 4 hr reflux. Esterification of homophthalic acid by *p*-TSA[3] gave monoester at the aliphatic part of the $-CO_2H$ group. Therefore, from the two different ^1H NMR spectra, the aromatic methyl ester (δ = 3.85 ppm) was subtracted from the aliphatic one. Thus, the regiospecific esterification of benzoic acid by Bpl was assured. According to the progress of the experiment, it is assumed that it will be a contending procedure with other existing methods because of its regiospecificity, high yields and simplicity(**Table I**).

Table 1-Analytical data of **1a-12a**

Entry	Acids	Reaction time (in hour)	Product (esters)	m.p. / b.p.*	Yield* (%)
1		4	 **1a**	b.p. 198-99 ^{0}C	88
2		5	 **2a**	b.p. 207-08^{0}C	86
3		5	 **3a**	49-50 ^{0}C	87
4		9	 **4a**	110-11 ^{0}C	77
5		7	 **5a**	b.p. 180-81 ^{0}C (Lit[10] b.p. 181^{0}C)	81
6		5	 **6a**	72-74 ^{0}C (Lit[10] 74^{0}C)	80
7		8	 **7a**	94-96 ^{0}C	78
8		10	 **8a**	b.p. 118-20 ^{0}C	52 (isolated)
9		12	 **9a**	36-38 ^{0}C	54 (isolated)
10		4	 **10a**	154-56 ^{0}C	88
11		4.5	 **11a**	136-37 ^{0}C	90
12		4.5	 **12a**	177-78 ^{0}C	86

*All the yields were calculated after silica gel filtration and melting / boiling points were checked from Aldrich chemical catalogue.

Experimental Section

The melting points were determined on a capillary melting point apparatus and are uncorrected. Infrared spectra were recorded using KBr pellets for solids and neat for liquids on FTIR-8400 and Perkin-Elmer 883 grating spectrometer. ^{1}H NMR and ^{13}C NMR spectra were taken on AC Bruker 200 MHz spectrometer in $CDCl_3$ containing TMS as the internal standard. Mass

spectra were taken on Kratos MS 80 system. All *J* values are given in Hz, chemical shifts in δ units. MeOH was dried over CaO. Reactions were monitored by TLC and column chromatography were carried out on 60-120 mesh E. Merck silica gel.

Extraction of Beef pancreas lipase (Bpl)

About 250 g of Beef Pancreas was taken and cut into small pieces. All the pieces were washed, dried by normal fan air and then made into dust by pestle. Dusts were placed into a round-bottomed flask with 100 mL methanol and stored for two days at normal temperature. Residue was separated by filtration and the filtrate was evaporated to dryness under reduced pressure using high vacuum pump. A light brown powder was collected.

General methods for the preparation of esters

To a stirred solution of benzoic acid (entry 1, 0.05g, 0.41 mmole) in 10 mL of dried methanol, catalytic amount of Bpl (5~7 mg) was added under ambient temperature. After stirring for 4 hr, a TLC was checked in pet.ether/ethyl acetate (5:1) solvent system. A new spot above the starting acid was detected. So, the bulk methanol was evaporated under reduced pressure and water (20 mL) was added to the residue. The separated oily layer was extracted with ether (3 × 15 mL), washed with brine (3 × 15 mL), dried over $MgSO_4$ and finally concentrated. Quick silica gel filtration separated the Bpl and gave the ester with excellent yields (see **Table I**).

Compound 10a. m.p. 154-156ºC, IR (KBr): 1720, 1643 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85 (d, 1H, *J* = 8 Hz, Ar-*H*), 7.49-7.42 (m, 2H, Ar-*H*), 7.21 (d, 1H, *J* = 8 Hz, Ar-*H*), 5.34 (s, 2H, -CH_2), 3.89 (s, 3H, Ar-CO_2CH_3).

Compound 11a. m.p. 136-137ºC, IR (KBr): 1735, 1698 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.43 (d, 1H, *J* = 2 Hz, Ar-*H*), 7.21 (d, 1H, *J* = 8 Hz, Ar-*H*), 7.02 (dd, 1H, *J* = 2.8 Hz), 4.80 (s, 2H, -CH_2), 3.89 (s, 3H, Ar-OCH_3), 3.79 (s, 3H, Ar-CO_2CH_3).

Compound 12a. m.p. 177-178ºC, IR (KBr): 1737, 1691 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36 (s, 1H, Ar-*H*), 7.04 (d, 1H, *J* = 8 Hz, Ar-*H*), 6.90 (dd, 1H, *J* = 4.8 Hz, Ar-*H*), 5.41 (s, 1H, Ar-O*H*), 4.01 (s, 2H), 3.85 (s, 3H, Ar-CO_2CH_3).

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