Novel Regioselective Ester Hydrolysis by Pig-Liver Esterase

Amit Basak,* Gautam Bhattacharya, and Sunanda K. Palit

Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, India

(Received October 16, 1996)

Pig-liver esterase, which catalyzed the hydrolysis of substrates containing both saturated and α,β-unsaturated/cyclopropanecarboxylic esters (methyl and ethyl), was studied. An exclusive hydrolysis of the saturated esters was observed. Kinetic experiments revealed that the presence of deactivated carbonyl in the unsaturated/cyclopropanecarboxylic esters and their weaker bindings are both responsible for the observed specificity. The relative binding abilities of the substrates have been explained in light of Jones active-site model. The regioselectivity has been exploited in the synthesis of intermediates for the thromboxane synthetase inhibitor.

The preferential transformation[1] of functional groups is a topic of considerable interest in organic synthesis. Strategies based on chemical reagents[2] or protecting groups[3] have been developed, depending on the nature of the functionalities. These chemical methods, however, have limitations, especially in the context of the degree of selectivity when compared to enzymatic processes.[4] Enzymes can work in organic solvents[5] and can distinguish between functional groups, electronically anisotropic or when they differ regio or stereochemically.[6] Pig-liver esterase (PLE) is one such enzyme, which has found enormous applications, particularly in the enantioselective hydrolysis of prochiral and meso diesters.[7] Among the several active-site models[8,9] for PLE, the cubic-space model proposed by Jones et al.[9] seems to be most useful. Apart from the interfacing pairs of hydrophobic and polar pockets, the another interesting feature of this model is the existence of a "gate" at the entrance to a large hydrophobic pocket (Fig. 1). The presence of the "gate" incorporates some restrictions on the steric disposition of groups near to the ester functionality. Steric constraints, e.g. double bonds or small rings near to the ester functionality, can be expected to slow down the rate of hydrolysis, and, thus, may lead to regioselective transformations.

In order to test our predictions, the kinetics of the hydrolysis of saturated methyl esters (**1** and **2**) as well as α,β-unsaturated and cyclopropanecarboxylic methyl esters (**3** and **4**) were studied. It was revealed that the four esters underwent hydrolysis at the following relative rates: $V_1 : V_2 : V_3 : V_4 \approx 70 : 85 : 1 : 2$.

These differences in the rates raised the possibility of the regioselective hydrolysis of esters in substrates containing both saturated and unsaturated/cyclopropanecarboxylic esters. Thus, substrates containing both methyl ester functionalities were prepared and subjected to PLE-catalyzed hydrolysis. In all cases, the saturated acyclic esters underwent hydrolysis while keeping the other intact (Scheme 1).[10,11] Neither the alternate hydrolysis product nor the diacid could be detected by 200 MHz NMR. Poor selectivity was observed when these substrates were saponified under normal conditions (1 M NaOH, EtOH, heat) (1 M = 1 mol dm^{-3}) or even under mild conditions (LiOH, H_2O–THF). Since both the methyl esters in substrates containing saturated and unsaturated esters had similar chemical shifts, the structures of the products from them had to be confirmed by preparing the benzyl esters and comparing them with authentic samples by NMR (Scheme 2). For those substrates containing both saturated and cyclopropanecarboxylic esters, the upfield methyl ester α to the cyclopropyl remained intact in the product. Additionally, the structures of the products were confirmed by ^{1}H NMR of the alcohols (**6c—9c**, **11c—15c**) obtained via the reduction of mixed anhydride prepared from the hydrolyzed products. The alcohols showed the characteristic pair of triplets at $\delta = 4.1$ and 4.3. Incidentally, the formation of alcohols (**6c—9c**, **11c—15c**) demonstrates the utility of the observed regioselectivity. Their imidazole derivatives are important for studying the inhibition of thromboxane (Tx A_2) synthetase.[12]

It may be argued that the observed selectivity could be entirely due to the reduced reactivity of the carbonyl carbon in unsaturated and cyclopropanecarboxylates; the binding phenomenon[13a] might not have any role. To determine the contribution of each factor, the relative K_{cat} and K_m values for various esters **1—4** were determined (Table 1).

Thus, the electronic factor does make a significant contribution to the observed specificity, as is reflected in the relative K_{cat} values. However, this is not the only discriminatory factor, considering the greater differences in rates. The weaker bindings of **3** and **4** (higher K_m's) are also partly responsible for the slower rate of hydrolysis. This is in agreement with our original assumption based on Jones model. Various substrates (**5a—15a**) bind preferably in such a fashion that the saturated ester functionality is placed at the catalytic site. The alternate binding mode, which will ensure the hydrolysis of the unsaturated/cyclopropanecarboxylic ester, is comparatively much weaker.[13b]

In conclusion, we have demonstrated a novel regioselec-

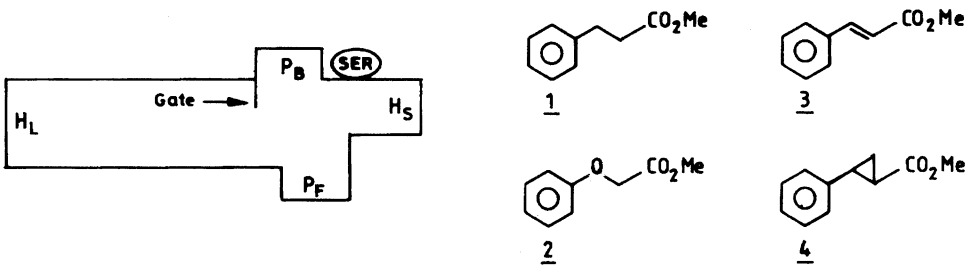
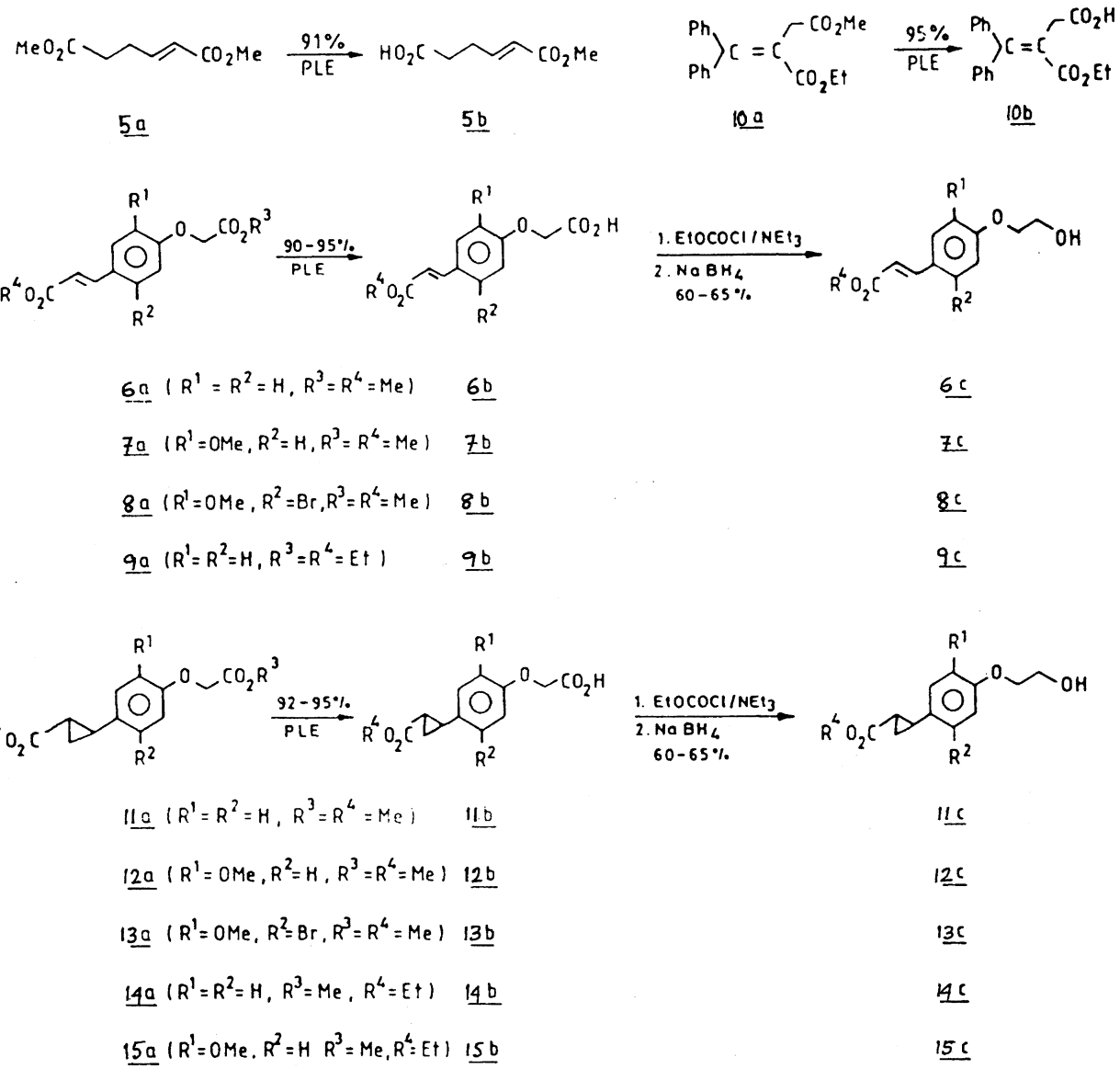
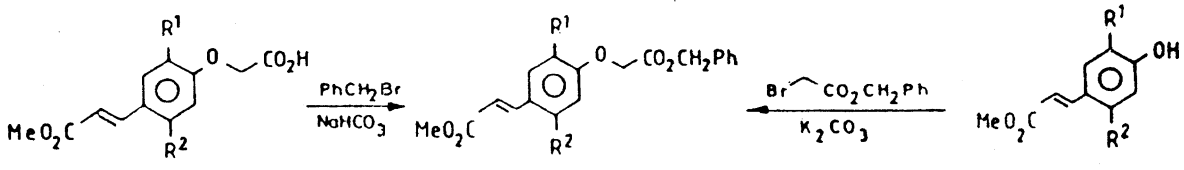


Fig. 1.



Scheme 1.



Scheme 2.

Table 1. Kinetic Parameters of Substrates **1**—**4** Undergoing PLE-Catalyzed Hydrolysis

Substrate	K_m (mmol)	Relative K_{cat}
1	5.9	12.4
2	5.3	15.6
3	33.3	1.0
4	19.2	1.1

tive hydrolysis of methyl/ethyl esters by PLE. The synthesis of intermediates for thromboxane synthetase inhibitors expands the scope of using such esters as protecting groups.

Experimental

PLE was isolated as acetone powder (200 units per g) from fresh pig liver according to a literature procedure.[7] Cell-free preparations were also made following a procedure developed by Seebach.[14] Hydrolytic reactions were carried out with both preparations, and the results were identical. NMR spectra were measured in a 200 MHz Bruker AC200 instrument, while the IR spectra were recorded on a Perkin–Elmer spectrophotometer. All of the compounds were fully characterized by spectral data. The NMR spectra were all recorded in $CDCl_3$. All recrystallizations were performed using a mixture of ethyl acetate and hexane.

Synthesis of Substrates. Dimethyl hex-2-enedioate **5a** was prepared from methyl 4-oxobutanoate via a Wittig reaction using methyl methyltriphenylphosphoranylideneacetate.[15] The diesters **6a**—**9a** were prepared from methyl or ethyl 4-hydroxyphenylacrylates by O-alkylation with methyl or ethyl bromoacetate in K_2CO_3 and DMF.[16] The diester **10a** was prepared via the Stobbe condensation[17] between benzophenone and diethyl succinate, followed by methylation (CH_3I, DMF, Na_2CO_3). Various cyclopropyl derivatives (**11a**—**15a**) were synthesized from the respective unsaturated esters by $Pd(OAc)_2$-catalyzed cyclopropanation with diazomethane.[18]

Dimethyl 2-Hexenedioate (5a). Yield 92%; viscous oil; IR ν_{max} (neat) 1705 cm^{-1}; ^1H NMR δ_H 2.47 (4H, m), 3.69 (3H, s), 3.75 (3H, s), 5.83 (1H, d, J = 16 Hz), 6.92 (1H, dt, J = 5.1, 16 Hz); ^{13}C NMR δ_C = 27.21, 32.20, 51.51, 121.80, 146.80, 173.2.

Methyl 3-[4-(Methoxycarbonylmethoxy)phenyl]acrylate (6a). Yield 95%; mp 94 °C; IR ν_{max} (KBr) 1735 cm^{-1}; ^1H NMR δ_H = 3.78 (3H, s), 3.80 (3H, s), 4.66 (2H, s), 6.31 (1H, d, J = 16 Hz), 6.90 (2H, d, J = 10 Hz), 7.48 (2H, d, J = 10 Hz), 7.58 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 51.58, 52.31, 65.22, 114.96, 116.07, 128.26, 129.73, 144.14, 159.43, 168.50, 169.50.

Methyl 3-[2-Methoxy-4-(methoxycarbonylmethoxy)phenyl]-acrylate (7a). Yield 94%; mp 81 °C; IR ν_{max} (KBr) 1729 cm^{-1}; ^1H NMR δ_H = 3.79 (6H, s), 3.91 (3H, s), 4.72 (2H, s), 6.32 (1H, d, J = 16 Hz), 6.79 (1H, d, J = 10 Hz), 7.01 (2H, m), 7.62 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 51.54, 62.20, 55.93, 66.09, 110.71, 113.69, 116.27, 121.96, 128.89, 144.36, 149.14, 149.70, 167.41, 168.92.

Methyl 3-[5-Bromo-2-methoxy-4-(methoxycarbonylmethoxy)phenyl]acrylate (8a). Yield 92%; mp 74°C; IR ν_{max} (KBr) 1728 cm^{-1}; ^1H NMR δ_H = 3.78 (6H, s), 3.88 (3H, s), 4.60 (2H, s), 6.30 (1H, d, J = 16 Hz), 6.90 (1H, d, J = 2 Hz), 7.25 (1H, d, J = 2 Hz), 7.35 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 50.58, 51.86, 36.34, 69.98, 110.68, 117.50, 129.19, 125.19, 132.54, 142.56, 146.02, 152.59, 166.69, 170.54. Found: C, 46.84; H, 4.21; Br, 22.26%. Calcd for $C_{14}H_{15}O_6Br$: C, 46.82; H, 4.20; Br, 22.25%.

Ethyl 3-[4-(Ethoxycarbonylmethoxy)phenyl]acrylate (9a). Yield 90%; mp 70 °C; IR ν_{max} (KBr) 1720 cm^{-1}; ^1H NMR δ_H = 1.22 (6H, t, J = 8 Hz), 4.22 (4H, overlapped q), 4.62 (2H, s), 6.28 (1H, d, J = 16 Hz), 6.87 (2H, d, J = 8 Hz), 7.44 (2H, d, J = 8 Hz), 7.60 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 14.18, 14.36, 60.50, 60.51, 64.65, 114.91, 116.53, 116.60, 129.74, 143.90, 149.00, 167.37, 172.64. Found: C, 64.70; H, 6.15%. Calcd for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52%.

Methyl 3-Ethoxycarbonyl-4,4-diphenyl-3-butenoate (10a). Yield 70%; viscous oil; IR ν_{max} (neat) 1735, 1705 cm^{-1}; ^1H NMR δ_H = 0.86 (3H, t, J = 8 Hz), 3.45 (2H, s), 3.71 (3H, s), 3.93 (2H, q, J = 8 Hz), 7.22 (10 H, m); ^{13}C NMR δ_C = 13.54, 29.70, 37.90, 51.98, 60.58, 125.18, 127.77, 127.88, 128.28, 128.41, 128.76, 129.15, 140.63, 142.21, 169.0, 171.49. Found: C, 74.18; H, 6.30%. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21%.

Methyl 2-[4-Methoxycarbonylmethoxy)phenyl]cyclopropanecarboxylate (11a). Yield 92%; mp 88 °C; IR ν_{max} (KBr) 1717 cm^{-1}; ^1H NMR δ_H = 1.20 (1H, m), 1.52 (1H, m), 1.81 (1H, m), 2.49 (1H, m), 3.65 (3H, s), 3.81 (3H, s), 4.61 (2H, s), 6.71 (2H, d, J = 8 Hz), 7.10 (2H, d, J = 8 Hz); ^{13}C NMR δ_C = 16.71, 23.67, 25.60, 31.89, 52.13, 65.0, 114.21, 127.68, 133.61, 156.20, 172.40, 173.60. Found: C, 63.82; H, 6.25%. Calcd for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10%.

Methyl 2-[2-Methoxy-4-(methoxycarbonylmethoxy)phenyl]-cyclopropanecarboxylate (12a). Yield 93%; mp 78 °C; IR ν_{max} (KBr) 1722 cm^{-1}; ^1H NMR δ_H = 1.30 (1H, m), 1.51 (1H, m), 1.80 (1H, m), 2.49 (1H, m), 3.65 (3H, s), 3.73 (3H, s), 3.79 (3H, s), 4.66 (2H, s), 6.67 (1H, dd, J=2, 8 Hz), 6.72 (1H, d, J=2 Hz), 6.75 (1H, d, J = 8 Hz); ^{13}C NMR δ_C = 16.64, 23.65, 25.98, 51.82, 52.08, 55.53, 66.66, 110.94, 114.76, 118.06, 134.43, 146.02, 149.67, 169.43, 173.72.

Methyl 2-[5-Bromo-2-methoxy-4-(methoxycarbonylmethoxy)phenyl]cyclopropanecarboxylate (13a). Yield 97%; mp 71 °C; IR ν_{max} (KBr) 1724 cm^{-1}; ^1H NMR δ_H = 1.25 (1H, m), 1.54 (1H, m), 1.82 (1H, m), 2.41 (1H, m), 3.68 (3H, s), 3.78 (6H, s), 4.55 (2H, s), 6.59 (1H, d, J = 2.1 Hz), 6.80 (1H, d, J = 2.1 Hz); ^{13}C NMR δ_C = 16.80, 23.70, 29.70, 51.30, 52.0, 56.10, 69.20, 110.60, 117.20, 122.10, 137.60, 143.30, 152.60, 169.0, 173.40. Found: C, 48.28; H, 4.50%. Calcd for $C_{15}H_{17}O_6Br$: C, 48.26; H, 4.59%.

Ethyl 2-[4-Methoxycarboxylmethoxy)phenyl]cyclopropanecarboxylate (14a). Yield 90%; mp; 87 °C; IR ν_{max} (KBr) 1722 cm^{-1}; ^1H NMR δ_H = 1.23 (1H, t, J = 7 Hz), 1.24 (1H, m), 1.52 (1H, m), 1.82 (1H, m), 2.40 (1H, m), 3.79 (3H, s), 4.16 (2H, q, J = 7 Hz), 4.60 (2H, s), 6.81 (2H, d, J = 8 Hz), 7.02 (2H, d, J = 8 Hz); ^{13}C NMR δ_C = 14.17, 16.64, 23.81, 25.44, 52.13, 60.36, 114.62, 127.39, 133.27, 156.44, 169.30, 173.34. Found: C, 64.62; H, 6.39%. Calcd for $C_{15}H_{18}O_5$: C, 64.74, H, 6.52%.

Ethyl 2-[2-Methoxy-4-(methoxycarboxylmethoxy)phenyl]cyclopropanecarboxylate (15a). Yield 91%; mp 71 °C; IR ν_{max} (KBr) 1722 cm^{-1}; ^1H NMR δ_H = 1.165 (1H, m), 1.20 (3H, t, J = 7 Hz), 1.50 (1H, m), 1.75 (1H, m), 2.40 (1H, m), 3.70 (3H, s), 3.80 (3H, s), 4.15 (2H, q, J = 7 Hz), 4.65 (2H, s), 6.55 (1H, d, J = 8 Hz), 6.62 (1H, s), 6.75 (1H, d, J = 8 Hz); ^{13}C NMR δ_C = 14.04, 16.54, 23.72, 25.66, 51.89, 55.69, 60.45, 66.40, 110.59, 114.49, 117.78, 134.35, 145.76, 149.45, 169.26, 173.09.

General Hydrolytic Procedure. A solution of the substrate (2 mmol) in phosphate-buffer (pH 7.2, 30 ml) and acetone (15 ml) was treated with PLE (200 mg) and stirred at ambient temperature for 10 h. It was filtered through celite, pH adjusted to 2 and partitioned between AcOEt and water. The organic layer was dried (Na_2SO_4), filtered and evaporated to afford the product as a while solid, crystallized from AcOEt–petroleum ether. The spectral characteristics are described below:

2-Hexenedioic Acid 1-Methyl Ester (5b). Yield 91%; viscous

oil; IR ν_{max} (neat) 1716 cm^{-1}; ^1H NMR δ_H=2.53 (4H, m), 3.70 (3H, s), 5.88 (1H, d, J = 10 Hz), 6.96 (1H, dt, J = 5.1, 16 Hz); ^{13}C NMR δ_C = 26.91, 30.89, 51.52, 122.13, 146.40, 166.68, 173.12.

[4- (2- Methoxycarbonylethenyl)phenoxy]acetic Acid (6b). yield 92%; mp 145 °C; IR ν_{max} (KBr) 1730 cm^{-1}; ^1H NMR δ_H = 3.74 (3H, s), 4.58 (2H, s), 4.93 (1H, br), 6.26 (1H, d, J = 16 Hz), 6.87 (2H, d, J = 8 Hz), 7.42 (2H, d, J = 8 Hz), 7.58 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 51.54, 64.89, 114.91, 115.70, 127.86, 129.67, 144.26, 159.52, 167.65, 170.58. Found: C, 61.25; H, 5.19%. Calcd for $C_{12}H_{12}O_5$: C, 61.02; H, 5.12%.

[3- Methoxy- 4- (2- methoxycarbonylethenyl)phenoxy]acetic Acid (7b). Yield 95%; mp 115 °C; IR ν_{max} (KBr) 1711 cm^{-1}; ^1H NMR δ_H = 3.80 (3H, s), 3.90 (3H, s), 4.18 (1H, br), 4.71 (2H, s), 6.35 (1H, d, J = 16 Hz), 6.81 (1H, d, J = 8 Hz), 7.05 (2H, m), 7.60 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 51.69, 55.96, 66.26, 110.78, 114.53, 116.52, 122.06, 129.35, 144.35, 148.85, 149.72, 167.59, 172.29. Found: C, 58.65; H, 5.35%. Calcd for $C_{13}H_{14}O_6$: C, 58.64; H, 5.30%.

[2- Bromo- 5- methoxy- 4- (2- methoxycarbonylethenyl)phenoxy]acetic Acid (8b). Yield 93%; mp 125 °C; IR ν_{max} (KBr) 1725 cm^{-1}; ^1H NMR δ_H = 3.80 (3H, s), 3.91 (3H, s), 4.66 (2H, s), 6.41 (1H, d, J = 16 Hz), 6.99 (1H, d, J = 1 Hz), 7.25 (1H, d, J = 1 Hz), 7.40 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 50.57, 56.34, 69.98, 110.67, 117.49, 125.20, 129.18, 132.50, 142.50, 146.0, 152.6, 166.7, 170.5. Found: C, 45.29; H, 3.84%. Calcd for $C_{13}H_{13}O_6Br$: C, 45.23; H, 3.80%.

[4- (2- Ethoxycarbonylethenyl)phenoxylacetic Acid (9b). Yield 93%; mp 105 °C; IR ν_{max} (KBr) 1719 cm^{-1}; ^1H NMR δ_H = 1.29 (3H, t, J = 7 Hz), 4.24 (2H, q, J = 7 Hz), 4.66 (2H, s), 6.31 (1H, d, J = 16 Hz), 6.89 (2H, d, J = 8 Hz), 7.47 (2H, d, J = 8 Hz), 7.63 (1H, d, J = 16 Hz); ^{13}C NMR δ_C = 14.27, 60.51, 64.54, 114.90, 128.43, 129.73, 143.89, 158.98, 167.36, 172.63.

3-Ethoxycarbonyl-4,4-diphenyl-3-butenoic Acid (10b). Yield 95%; mp 121 °C; lit,[19b] 125 °C; IR ν_{max} 1701 cm^{-1}; ^1H NMR δ_H = 0.87 (3H, t, J = 8 Hz), 3.35 (2H, s), 3.97 (2H, q, J = 8 Hz), 7.14 (10H, m); ^{13}C NMR δ_C = 13.35, 37.92, 60.81, 124.35, 127.89, 128.37, 128.46, 128.61, 128.98, 140.43, 141.39, 152.55, 169.33, 171.12. Found: C, 73.73; H, 5.90%. Calcd for $C_{19}H_{18}O_4$: C, 73.53; H, 5.85%.

[4-(2-Methoxycarbonylcyclopropyl)phenoxy]acetic Acid (11b). Yield 95%; mp 91 °C; IR ν_{max} (KBr) 1720 cm^{-1}; ^1H NMR δ_H = 1.23 (1H, m), 1.53 (1H, m), 1.79 (1H, m), 2.46 (1H, m), 3.66 (3H, s), 4.63 (2H, s), 6.83 (2H, d, J = 8 Hz), 7.02 (2H, d, J = 8 Hz); ^{13}C NMR δ_C = 16.74, 23.75, 25.65, 51.48, 64.97, 114.83, 127.70, 133.56, 156.38, 173.20, 173.65. Found: C, 62.48; H, 5.67%. Calcd for $C_{13}H_{14}O_5$: C, 62.39; H, 5.64%.

[3-Methoxy-4-(2-methoxycarbonylcyclopropyl)phenoxy]acetic Acid (12b). Yield 92%; mp 83 °C; IR ν_{max} (KBr) 1715 cm^{-1}; ^1H NMR δ_H = 1.32 (1H, m), 1.60 (1H, m), 1.82 (1H, m), 2.47 (1H, m), 3.69 (3H, s), 3.74 (3H, s), 4.67 (2H, s), 6.68 (1H, dd, J=2, 8 Hz), 6.72 (1H, d, J = 2 Hz), 6.75 (1H, d, J = 8 Hz); ^{13}C NMR δ_C = 16.64, 23.65, 25.98, 52.08, 55.52, 66.65, 110.94, 114.76, 118.06, 134.43, 146.02, 169.72, 173.72. Found: C, 59.85; H, 5.71%. Calcd for $C_{14}H_{16}O_6$: C, 60.00; H, 5.75%.

[2- Bromo- 5- methoxy- 4- (2- methoxycarbonylcyclopropyl)-phenoxy]acetic Acid (13b). Yield 93%; mp 102 °C; IR ν_{max} (KBr) 1720 cm^{-1}; ^1H NMR δ_H = 1.30 (1H, m), 1.61 (1H, m), 1.82 (1H, m), 2.43 (1H, m), 3.63 (3H, s), 3.80 (3H, s), 4.58 (2H, s), 6.65 (1H, bs), 6.85 (1H, bs); ^{13}C NMR δ_C = 16.71, 23.80, 25.52, 52.05, 56.25, 70.28, 110.37, 116.93, 122.39, 138.51, 143.26, 152.24, 170.46, 173.32. Found: C, 46.42; H, 4.15%. Calcd for $C_{14}H_{15}O_6Br$: C, 46.82; H, 4.20%.

[4-(2-Ethoxycarbonylcyclopropyl)phenoxy]acetic Acid (14b). Yield 96%; mp 103 °C; IR ν_{max} (KBr) 1719 cm^{-1}; ^1H NMR δ_H = 1.22 (3H, t, J = 7 Hz), 1.24 (1H, m), 1.53 (1H, m), 1.82 (1H, m), 2.50 (1H, m), 4.16 (2H, q, J = 7 Hz), 4.64 (2H, s), 6.84 (2H, d, J = 8 Hz), 7.05 (2H, d, J = 8 Hz); ^{13}C NMR δ_C = 14.17, 16.64, 23.80, 25.44, 60.56, 65.35, 114.62, 127.94, 133.26, 156.44, 169.27, 173.33. Found:C, 63.43; H, 6.02%. Calcd for $C_{14}H_{16}O_5$: C, 63.63, H, 6.10%.

[4-(2-Ethoxycarbonylcyclopropyl)-3-methoxyphenoxy]acetic Acid (15b). Yield 96%; mp 101 °C; IR ν_{max} (KBr) 1726 cm^{-1}; ^1H NMR δ_H = 1.17 (1H, m), 1.19 (3H, t, J = 7 Hz), 1.50 (1H, m), 1.82 (1H, m), 2.38 (1H, m), 3.78 (3H, s), 4.06 (2H, q, J = 7 Hz), 4.57 (2H, s), 6.58 (1H, d, J = 8 Hz), 6.59 (1H, d, J = 8 Hz), 6.70 (1H, d, J = 8 Hz), 9.15 (1H, br); ^{13}C NMR δ_C = 14.16, 16.77, 23.91, 25.66, 55.88, 60.81, 66.95, 110.81, 115.59, 118.17, 135.50, 145.75, 149.44, 172.91, 173.09. Found: C, 61.19; H, 6.28%. Calcd for $C_{15}H_{18}O_6$: C, 61.22; H, 6.16%.

General Procedure for $NaBH_4$ Reduction. Triethylamine (0.55 mmol) was added to a stirring solution of the monocarboxylic acid (**6b—9b, 11b—15b**) (0.5 mmol) in THF (10 ml) under N_2 at −5 °C; the mixture was stirred for 10 min. Ethyl chloroformate (0.55 mmol) in THF (2 ml) was added and stirred for a further 5 min. The solution was filtered and the filtrate was slowly added to a solution of $NaBH_4$ in THF (5 ml) and stirred at room temperature for 10 h. The products were extracted into ethyl acetate and the organic layer was evaporated to leave an oily residue, from which the alcohols (**6c—9c, 11c—15c**) were isolated in a pure form by chromatography. Their ^1H NMR data are mentioned:

Methyl 3-[4-Hydroxyethoxy)phenyl]acrylate (6c). ^1H NMR δ_H = 3.81 (3H, s), 3.98 (2H, t, J = 5 Hz), 4.18 (2H, t, J = 5 Hz), 6.30 (1H, d, J = 16 Hz), 6.90 (2H, d, J = 8 Hz), 7.48 (2H, d, J = 8 Hz), 7.62 (1H, d, J = 16 Hz).

Methyl 3-[4- (2-hydroxyethoxy)- 2-methoxyphenyl]acrylate (7c). ^1H NMR δ_H = 3.80 (3H, s), 3.90 (3H, s), 3.97 (2H, t, J = 5 Hz), 4.26 (2H, t, J = 5 Hz), 6.32 (1H, d, J = 16 Hz), 6.88 (1H, d, J = 8 Hz), 7.04 (2H, m), 7.62 (1H, d, J = 16 Hz).

Methyl 3-[5-Bromo-4-(2-hydroxyethoxy)-2-methoxyphenyl]-acrylate (8c). ^1H NMR δ_H = 3.78 (3H, s), 3.91 (3H, s), 3.98 (2H, t, J = 5.1 Hz), 4.27 (2H, t, J = 5.1 Hz), 6.32 (1H, d, J = 16 Hz), 6.91 (1H, d, J = 2 Hz), 7.26 (1H, d, J = 2 Hz), 7.35 (1H, d, J = 16 Hz).

Ethyl 3-[4-(2-Hydroxyethoxy)phenyl]acrylate (9c). ^1H NMR δ_H = 1.23 (3H, t, J = 7.2 Hz), 3.90 (3H, s), 3.98 (2H, t, J = 5 Hz), 4.22 (2H, q, J = 7.2 Hz), 4.26 (2H, t, J = 5 Hz), 6.30 (1H, d, J = 16 Hz), 6.91 (1H, d, J = 8 Hz), 7.49 (2H, m), 7.58 (1H, d, J = 16 Hz).

Methyl 2-[4-(2-Hydroxyethoxy)phenyl]cyclopropanecarboxylate (11c). ^1H NMR δ_H = 1.16 (1H, m), 1.55 (1H, m), 1.92 (1H, m), 2.48 (1H, m), 3.65(3H, s), 3.94 (2H, t, J = 5 Hz), 4.11 (2H, t, J = 5 Hz), 6.72 (2H, d, J = 8 Hz), 7.14 (2H, d, J = 5 Hz).

Methyl 2-[4-(2-Hydroxyethoxy)-2-methoxyphenyl]cyclopropanecarboxylate (12c). ^1H NMR δ_H = 1.30 (1H, m), 1.54 (1H, m), 1.82 (1H, m), 2.43 (1H, m), 3.67 (3H, s), 3.79 (3H, s), 3.95 (2H, t, J = 5.2 Hz), 4.12 (2H, t, J = 5.2 Hz), 6.82 (1H, d, J = 8 Hz), 7.11 (2H, m).

Methyl 2-[5-Bromo-4-(2-hydroxyethoxy)-2-methoxyphenyl]-cyclopropanecarboxylate (13c). ^1H NMR δ_H = 1.25 (1H, m), 1.54 (1H, m), 1.82 (1H, m), 2.41 (1H, m), 3.68 (3H, s), 3.79 (3H, s), 3.96 (2H, t, J = 5.1 Hz), 4.11 (2H, t, J = 5.1 Hz), 6.60 (1H, d, J = 2.1 Hz), 6.81 (1H, d, J = 2.1 Hz).

Ethyl 2-[4-(2-Hydroxyethoxy)phenyl]cyclopropanecarboxylate (14c). ^1H NMR δ_H = 1.23 (3H, t, J = 7 Hz), 1.25 (1H, m), 1.54 (1H, m), 1.83 (1H, m), 2.42 (1H, m), 3.79 (3H, s), 3.97 (2H, t, J = 5 Hz), 4.12 (2H, t, J = 5 Hz), 4.16 (2H, q, J = 7 Hz), 6.81 (2H, d,

$J = 8$ Hz), 7.04 (2H, d, $J = 8$ Hz).

Ethyl 2-[4-(2-Hydroxyethoxy)-2-methoxyphenyl]cyclopropanecarboxylate (15c). ^{1}H NMR $\delta_H = 1.16$ (1H, obscured), 1.26 (3H, q, $J = 7$ Hz), 1.55 (1H, m), 1.92 (1H, m), 2.48 (1H, m), 3.86 (3H, s), 3.93 (2H, t, $J = 5$ Hz), 4.11 (2H, t, $J = 5$ Hz), 4.16 (2H, q, $J = 7$ Hz), 6.63 (1H, $J = 7$ Hz), 6.94 (2H, m).

Kinetic Measurements: The esters (**1**—**4**) in various concentrations (0.04, 0.026, 0.013, and 0.007 M) were dissolved in phosphate buffer–acetone (3 : 1, pH 7.2). After an enzyme (200 mg, Fluka) was added, the mixture was stirred at 27 °C. The pH was kept constant at 7.2 by the addition of 0.1 M KOH at different time points. The amounts of free acid liberated were plotted against time. The initial velocities were calculated from the gradient of the resulting curves at the beginning of the reaction. The various K_m values were calculated from the double-reciprocal plots ($1/V_o$ vs. $1/S$. The relative K_{cat} values were obtained from the ratio of the V_{max} parameters.

The author thanks DST for financial assistance; GB thanks CSIR for a fellowship.

References

1) W. Bartman and B. M. Trost, "Selectivity—A goal towards synthetic efficiency," Verlag Chemie (1984).

2) D. C. Sarkar, A. R. Das, and B. C. Ranu, *J. Org. Chem.*, **55**, 5799 (1990); D. E. Ward, C. K. Rhee, and W. M. Zoghaib, *Tetrahedron Lett.*, **29**, 517 (1988).

3) T. W. Greene and P. G. M. Wets, "Protecting Groups of Organic Synthesis," Wiley Interscience, New York (1991).

4) W. Boland, C. Frobl, and M. Lorenz, *Synthesis*, **1991**, 1049.

5) A. Zaks and A. M. Klibanov, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 3192 (1985).

6) E. Santeniello, P. Ferraboschi, P. Grisonti, and A. Monzocchi, *Chem. Rev.*, **1992**, 1071.

7) K. Adachi, S. Kobayashi, and M. Ohno, *Chimia*, **40**, 311 (1986).

8) P. Mohr, N. Waespe-Sarcevie, C. Tamm, K. Gawronska, and J. K. Gawronski, *Helv. Chim. Acta*, **66**, 2501 (1983); M. Ohno, in "Enzymes in Organic Synthesis," Ciba Foundation Symposium III, ed by R. Clark and S. Porter, Pitman, London (1985), p. 171; F. Bjorkling, J. Boutelje, S. Gatenbeck, K. Hult, T. Norin, and P. Szmulik, *Tetrahedron*, **41**, 1347 (1985).

9) L. Provencher and J. B. Jones, *J. Org. Chem.*, **59**, 2729 (1994); L. Provencher, H. Wynn, J. B. Jones, and A. Krawezyk, *Tetrahedron Asymmetry*, **4**, 2025 (1993); E. J. Toone, M. J. Werth, and J. B. Jones, *J. Am. Chem. Soc.*, **112**, 4946 (1990).

10) A preliminary work on selective hydrolysis of saturated esters in the presence of unsaturated esters residing in different molecules has earlier been published: A. Basak, A. Nag, S. Panchal, and G. Bhattacharya, *Biotechnol. Lett.*, **15**, 19 (1993).

11) Chymotrypsin and papain can discriminate between saturated and unsaturated esters: C. Shin, M. Seki, and N. Takahashi, *Chem. Lett.*, **1990**, 2089.

12) K. Iizuka, K. Akahane, D. Momose, M. Nakazawa, T. Tanouchi, M. Kawamura, I. Ohyama, I. Kajiwara, Y. Iguchi, T. Okada, K. Taniguchi, T. Miyamoto, and M. Hayashi, *J. Med. Chem.*, **24**, 1139 (1981).

13) a) A. Fersht, "Enzyme Structure and Mechanism," 2nd ed, W. H. Freeman and Co., New York (1985); b) We have measured the K_M and relative K_{cat} values for the substrates **6a** and **11a**. Their K_M's are around 50 mmolar while K_{cat}'s are 10^2 order less compared to simple unsaturated or cyclopropyl esters **3** or **4** respectively.

14) D. Seebach and M. Eberle, *Chimia*, **40**, 315 (1986).

15) R. Adams, A. H. Blatt, V. Boekelheide, T. L. Cairns, A. C. Cope, and C. Niemann, "Organic Reactions," Vol. 14, p. 270—490 (1967).

16) G. Veriot, J. Dutasta, G. Matouzenko, and A. Collet, *Tetrahedron*, **50**, 390 (1994).

17) R. Adams, H. Adkins, A. H. Blatt, A. C. Cope, F. C. McGrew, C. Niemann, and H. R. Snyder, "Organic Reactions," Vol. 6, p. 1—73 (1964).

18) M. Suda, *Synthesis*, **1981**, 714.

19) W. S. Johnson, J. W. Peterson, and W. P. Schneider, *J. Am. Chem. Soc.*, **69**, 74 (1947).