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Effect of the α-Methyl Substituent on Chemoselectivity in Esterase-Catalyzed Hydrolysis of *S*-Acetyl Sulfanylalkanoates

Ish Kumar and Ravinder S. Jolly*

Department of Chemistry, Institute of Microbial Technology, Sector 39, Chandigarh 160 036, India

jolly@lion.imtech.ernet.in

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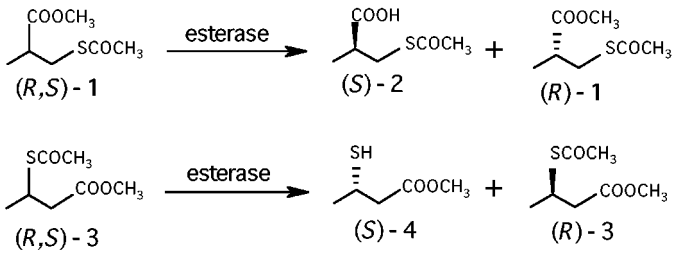
ABSTRACT



The isomeric compounds 1 and 3, which differ only in the position of a methyl substituent, give opposite chemoselectivities in an esterase-catalyzed hydrolysis reaction. The esterase was chemoselective for the oxoester in 1, but for the thiol ester group in 3. A high enantioselectivity was observed for both 1 and 3.

The catalytic use of enzymes in the hydrolysis of compounds containing both thiol ester and oxoester functional groups has been the subject of recent studies,[1−5] primarily because of the interest in the preparation of optically pure (*S*)-3-acetylsulfanyl-2-methylpropanoic acid (**2**), a key intermediate for[1] the antihypertensive drug captopril.[6] In addition, both the enantiomers of 2-sulfanylpropanoic acid (**6**) have found use in pharmaceutical and other applications.[7] A general knowledge of the effect of structure on the relative rates of hydrolysis of thiol esters and oxoesters, however, is not available. Bianchi and Cesti, for instance, studied several lipases for the hydrolysis of methyl 3-acetylsulfanyl-2-methylpropanoate (**1**). In all the cases, both ester groups were hydrolyzed, but the thiol ester group appeared to be more

(1) Hof, P. H.; Kellogg, R. M. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1247−1249.

(2) Bianchi, D.; Cesti, P. J. *J. Org. Chem.* **1990**, *55*, 5657−5659.

(3) Sakimae, A.; Hosoi, A.; Kobayashi, E.; Ohsuga, N.; Numazawa, R.; Watanabe, I.; Ohnishi, H. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 1252−1256.

(4) Sproull, K. C.; Bowman, G. T.; Carta, G.; Gainer, J. L. *Biotechnol. Prog.* **1997**, *13*, 71−76.

(5) For leading reviews on biocatalyzed hydrolysis reaction in general, see: (a) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071−1140. (b) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114−120. (c) Yamada, H.; Shimizu, S. *Angew. Chem., Int. Ed.* **1988**, *27*, 622−642. (d) Simon, H.; Bader, J.; Gunther, H.; Neumann, S.; Thanos, J. *Angew. Chem., Int. Ed.* **1985**, *24*, 539−553. Also see: Um, P. J.; Drueckhammer, D. G. *J. Am. Chem. Soc.* **1998**, *120*, 5605−5610, and references therein.

(6) Ondetti, M. A.; Cushman, D. W. *CRC Crit. Rev. Biochem.* **1984**, *16*, 381−411. Shimazaki, M.; Hasegawa, J.; Kan, K.; Nomura, K.; Nose, Y.; Kondo, H.; Ohashi, T.; Watanabe, K. *Chem. Pharm. Bull.* **1982**, *30*, 3139−3146. Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441−444.

(7) For examples, see the following. Analogue of Ala-82 in the backbone of T4 lysozyme: (a) Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197−200. Ultrashort acting ACE inhibitor: (b) Baxter, A. J. G.; Carr, A. D.; Eyley, S. C.; Fraser-Reid, L.; Hallam, C.; Herper, S. T.; Hurved, P. A.; King S. J.; Megani, P. *J. Med. Chem.* **1992**, *35*, 3718−3720. Platelet activating factor receptor antagonists: (c) Tanabe, Y. JP 0495 092/1992; *Chem. Abstr.* **1993**, *118*, 234049e. Tanabe, Y.; Suzukamo, G.; Komuro, S.; Imaishi, S.; Morooka, S.; Enomoto, M.; Kojima, A.; Sanemitsu, Y.; Mizutani, M. *Tetrahedron Lett.* **1991**, *32*, 379−382. In anti ulcer agents: (d) Masai, N.; Enomoto, M.; Kojima, A.; Masumori, H.; Hara, N.; Hara, Y.; Morooka, S. EP Appl. 325496/1989; *Chem. Abstr.* **1990**, *113*, 11529u.

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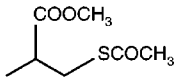
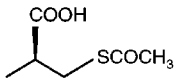
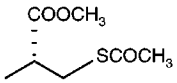
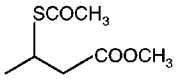
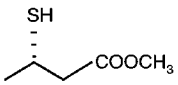
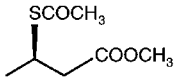
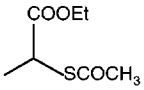
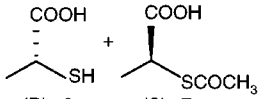
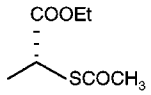
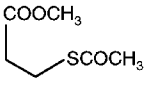
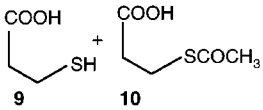
Table 1. Esterase-Catalyzed Hydrolysis of *S*-Acetyl Sulfanylalkanoates

Entry	Substrate	Conversion(%) (Time; hr)	Hydrolyzed Product	Yield(%)	ee(%)	Recovered Ester	Yield(%)	ee(%)[a]
1	 **1**	50 (0.3)	 *(S)* - **2**	48	99	 *(R)* - **1**	50	99
2	 **3**	50 (1.25)	 *(S)* - **4**[b]	45	99	 *(R)* - **3**[b]	47	100
3	 **5**	90 (2.5)	 *(R)* - **6** *(S)* - **7**	80 **6/7** = 4	**6**; 7% **7**; 21%	 *(R)*- **5**	5	-
4	 **8**	100 (6.0)	 **9** **10**	95 **9/10** = 10	-	-	-	-

[a] Determined as described in the text. [b] Absolute configuration not determined; the assignment of configuration is tentative.

reactive.[2] In enzymatic hydrolysis experiments with ethyl 2-acetylsulfanyl propanoate (**5**) as the substrate, PPL or *Pseudomonas* sp. lipase preferably hydrolyzed the thiol ester group,[2] whereas pig liver esterase preferred the oxoester group.[1]

Recently, we have isolated a new esterase from *Pseudomonas fluorescens* MTCC B0015.[8] We studied this esterase for the hydrolysis of various *S*-acetyl sulfanylalkanotes, viz., methyl 3-acetylsulfanyl-2-methylpropanoate (**1**), methyl 3-acetylsulfanylbutyrate (**3**), ethyl 2-acetylsulfanylpropanoate (**5**), and methyl 3-acetylsulfanylpropanoate (**8**). We observed a strong dependence of chemoselectivity on the presence of an α-methyl substituent. The esterase was found to have opposite chemoselectivities for isomeric compounds **1** and **3**; it was selective for the oxoester group in **1** but for the thiol ester group in **3**. A high enantioselectivity was also observed for both of these substrates. However, a very poor chemo- as well as enantioselectivity was obtained in the case of **5**. Here, we report the results of these investigations.

We started our investigations by studying the esterase-catalyzed hydrolysis[9,10] of **1** (entry 1 in Table 1) in[2] phosphate buffer at pH 6.8 and 37 °C. A rapid reaction occurred as evidenced by the drop in pH. The pH of the reaction mixture was maintained by the addition of 0.1 N NaOH. The reaction was stopped when there was no further drop in pH. The workup[9] followed by flash chromatography (silica gel; 1:1 CH_2Cl_2/ethyl acetate) gave the hydrolyzed product (**2**) and unreacted ester (**1**). The ^{1}H NMR spectral data of **2** revealed a peak at δ 2.35, indicating that the thiol ester group has remained intact, whereas the resonance peak due to the methyl ester group was absent. Its optical rotation corresponded with that of the *S* enantiomer. The e.e. was determined to be 99% on the basis of HPLC and NMR analysis of its derivative prepared by the condensation with (*R*)-(+)-1-(1-naphthyl)ethylamine.[3] The e.e. of unreacted ester was determined to be 99% by the ^{13}C satellite/Eu(hfc)$_3$ method.[11]

Next, we studied the hydrolysis of **3** (entry 2 in Table 1) under the above reaction conditions. Again, a rapid reaction was observed as indicated by the drop in pH. The reaction was stopped when there was no further drop in pH. The workup followed by flash chromatography (silica gel; 1:10 ethyl acetate/hexane) gave methyl 3-sulfanylbutyrate (**4**)[12]

(8) Preparation of esterase will be published elsewhere.

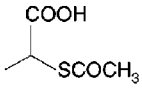
(9) **Representative Experiment**. A suspension of **1** (0.440 g, 2.5 mM) in phosphate buffer (pH 7.00, 50 mL) was purged with a steam of nitrogen for 5 min, esterase (150 IU) was added, and the contents were stirred vigorously; the pH of the solution was kept at 7.00 by continuos addition of 0.1 N aqueous NaOH. After 0.3 h, when there was no further drop in pH, the reaction mixture after acidification to pH 2.00 with dilute HCl was extracted with ether (3 × 10 mL). The organic extracts were washed with brine, dried (sodium sulfate), and then evaporated to give a mixture of acid **2** and the unchanged ester **1**. The separation of acid and ester was effected by flash chromatography.

(10) Compounds **1**, **2**, **5**−**7**, **9**, and **10** are known compounds.[1−3] ^{1}H NMR for compound **3** (300 MHz, $CDCl_3$): δ 1.36 (d, 3H, J = 7.5 Hz), 2.30 (s, 3H), 2.54 (dd, 1H, J = 7.5, 15.9 Hz), 2.68 (dd, 1H, J = 6.0, 15.9 Hz), 3.69 (s, 3H), 3.90 (m, 1H). ^{1}H NMR for compound **4** (300 MHz, $CDCl_3$): δ 1.40 (d, 3H, J = 6.8 Hz), 1.44 (d, 1H, J = 7.3 Hz), 2.54 (dd, 1H, J = 8.3, 15.9 Hz), 2.92 (dd, 1H, J = 6.1, 15.9 Hz), 3.51 (m, 1H), 3.70 (s, 1H). ^{1}H NMR for compound **8** (300 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.64 and 3.12 (each t, each 3H, J = 6.9 Hz), 3.68 (s, 3H).

(11) Strijtveen, B.; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664−3671, and references therein.

(12) Thiol **4** was found to be very unstable and is rapidly converted into disulfide. It must therefore be isolated with care.

Table 2. Esterase-Catalyzed Hydrolysis of 2-Sulfanylpropanoates

Entry	Substrate	Conversion (%)	Time (hr)	Product	Yield (%)	ee(%)[a]
1.	(*RS*) - **5**	30	0.7	(*S*) - **6** + (*S*) - **7**	94[b]; **6**/**7** = 4	**6**,10; **7**, 32
2.	 (*R*,*S*) - **11**	100	2.0	 (*R*,*S*) - **6**	99	0
3.	 (*R*,*S*) - **7**	0	20	NO REACTION	-	-
4.	(*R*) - **5**	100	3.0	(*R*) - **6** + (*R*) - **7**	95; **6**/**7** = 5.4	**6**, 96; **7**, 98
5.	(*S*) - **5**	100	2.4	(*S*) - **6** + (*S*) - **7**	95; **6**/**7** = 2.4	**6**, 95; **7**, 97

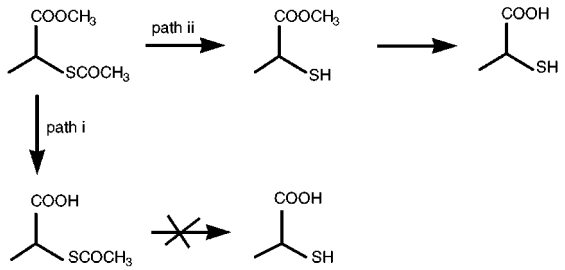
[a] Determined by comparison of optical rotations. [b] Based on recovered ester, which was enriched in the *R* enantiomer.

as the faster moving compound. Its enantiomeric excess was determined to be 99% on the basis of the ^{31}P NMR method.[11] The slower moving compound was unreacted ester. Its enantiomeric excess was determined to be 100% on the basis of the ^{13}C satellite/Eu(hfc)$_3$ method.[11]

In light of the opposite chemoselectivity observed for isomeric substrates **1** and **3**, we thought it would be interesting to study the hydrolysis of **5** (entry 3 in Table 1) in which a methyl group is α to both the thiol ester and the oxoester group. The reaction was stopped when there was no further drop in pH. The workup followed by flash chromatography (silica gel; 1:3 ethyl acetate/hexane) gave two compounds. The faster moving compound was identified as 2-sulfanylpropanoic acid (**6**). Its optical rotation corresponded with the *R* enantiomer (7% ee). The slower moving compound was identified as 2-acetylsulfanylpropanoic acid (**7**). Its optical rotation corresponded with the *S* enantiomer (21% ee).

A set of experiments was conducted to obtain some[4] understanding of the chemo- and enantioselectivity of the esterase-catalyzed hydrolysis for this substrate. The results of these experiments are summarized in Table 2. The following conclusions may be drawn from these results. (i) The esterase has a preference for the *S* enantiomer for both thiol ester and oxoester as is evident in the enantiomeric excess of the products obtained, when the reaction was stopped at 30% conversion (entry 1 in Table 2). (ii) Racemic ethyl 2-sulfanylpropanoate (**11**) gave racemic **6**, indicating a lack of any enantioselectivity for this substrate (entry 2 in Table 2). (iii) Racemic 2-acetylsulfanylpropanoic acid (**7**) was not hydrolyzed under the reaction conditions (entry 3 in Table 2). (iv) A product ratio of 1:5.4 obtained when pure (*R*)-**5** was used as the substrate (entry 4 in Table 2) and 1:2.4 when pure (*S*)-**5** was used as the substrate (entry 5 in Table 2) indicated that the thiol ester reacts faster than oxoester. (v) The hydrolysis follows the pathway shown in Scheme 1. 2-Acetylsulfanylpropanoic acid (**7**) produced initially by

Scheme 1



the competitive hydrolysis of oxoester (path i) remains intact and is not converted further into 2-sulfanylpropanoic acid (**6**). All the 2-sulfanylpropanoic acid (**6**) produced in the reaction therefore comes via path ii.

Finally, we studied the hydrolysis of **8** (entry 4 in Table 1), in which both the thiol ester and the oxoester lacked the α-methyl substituent. As expected, the hydrolysis of this compound occurred at a relatively reduced rate. Both the ester groups were hydrolyzed to give 3-sulfanylpropanoic acid (**9**) and 3-acetylsulfanylpropanoic acid (**10**).

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