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BIFUNCTIONAL CHIRAL SYNTHONS VIA BIOCHEMICAL METHODS. VIII. OPTICALLY-ACTIVE 3-AROYLTHIO-2-METHYLPROPIONIC ACIDS.[1]

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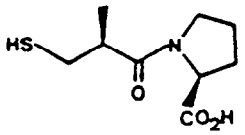
Summary: Optically-active 3-aroylthio-2-methylpropionic acids have been prepared via lipase-catalyzed enantiospecific hydrolysis of their corresponding esters.

Capoten, 1-[(2S)-3-mercapto-2-methylpropionyl]-L-proline (1), is the first member of a new class of antihypertensive agents effective in the management of heart failure.[2]



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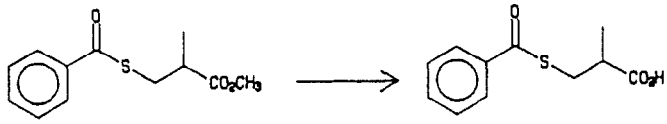
Although its mechanism of action has not yet been fully defined, it appears to act primarily via the suppression of the renin-angiotensin-aldosterone system.[2] Capoten, 1, is a specific competitive inhibitor of angiotensin I-converting enzyme (ACE), the enzyme responsible for the conversion of angiotensin I to angiotensin II.[3] However, the potency of the inhibitor of ACE depends critically on the configuration of the mercaptoalkanoyl moiety; the compound with the S-configuration is about 100 times more active than its corresponding R-enantiomer.[4] Capoten, 1, may be synthesized by coupling optically-active 3-acetylthio or 3-benzoylthio-2S-methylpropionic acid to L-proline, followed by deacylation of the product. In turn, the optically-active 3-acylthio-2-methylpropionic acids may be prepared in moderate yields via a rather expensive conventional chemical resolution procedure.[5] Alternatively, the requisite 3-mercapto-2S-methylpropionic acid may be synthesized from 3-hydroxy-2R-methylpropionic acid, which is obtained via a relatively expensive bacterial fermentation of isobutyric acid.[6] We herein describe a new method of preparing optically-active 3-aroylthio-2-methylpropionic acids via lipase-catalyzed hydrolysis of (±)-3-aroylthio-2-methylpropionic esters.

In a preliminary experiment, we noted that a statistical mixture of hydrolytic products was formed after exposure of (±)-methyl-3-acetylthio-2-methylpropionate to microbial lipases. Therefore, we perceived that the feasibility of this enzymatic approach depends on our ability to achieve the chemoselective cleavage of the alkoxy ester in the presence of the acylthio ester. Because benzoate esters are generally cleaved at a very slow rate

by most microbial lipases, we surmised that (±)-methyl-3-benzoylthio-2-methylpropionate may be a more suitable substrate to attain the required chemoselectivity. The results of Table 1 are in accord with our notion. They clearly show that the benzoylthio ester is indeed resistant to attack by all the lipases examined. Although the lipase of *Aspergillus niger* is uniquely enantiospecific (E = >100), it preferentially cleaved the undesired R-enantiomer. Unfortunately, all the lipases that possessed the desired S-stereochemical preference (*Mucor* and *Rhizopus*), exhibited rather low enantiospecificity (E = 2-3).

TABLE 1. Enantiospecific Hydrolysis of (±)-Methyl-3-benzoylthio-2-methylpropionate (2) by Microbial Lipases.



Lipase Source[1]	Stereochemical Preference	Extent of Conversion (%)	Enantiomeric Excess (%) Ester	Acid	E
Aspergillus niger[1a]	R	32	45	98	>100[2]
Pseudomonas sp.[1b]	R	39	52	81	16
Chromobacterium violaceum[1c]	R	41	52	75	12
Mucor meihei[1d]	S	37	26	43	3
Rhizopus niveus[1e]	S	15	6	33	2
Rhizopus arrhizus[1f]	S	32	11	24	2

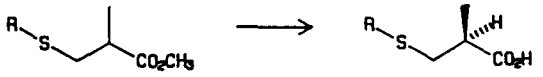
[1] To one ml of 0.2 M phosphate buffer, pH 7.0, was added 238 mg (1 mmol) of (±)2 and varying amounts of different enzyme preparations. The contents were incubated at 22°C for 44 h under gentle stirring. [a]50 mg of Amano K-10 powder; [b]20 mg of Amano LPL-80; [c]1 mg of U.S. Biochemicals, 3,400 u/mg; [d]40 mg of Amano MAP-10 powder; [e]50 mg of Amano-N powder; [f]1 mg of enzyme of Boehringer-Mannheim.

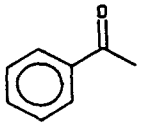
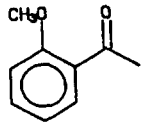
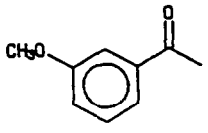
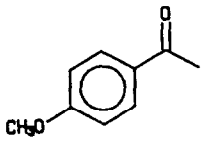
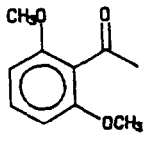
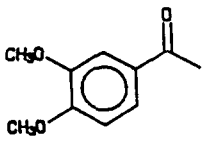
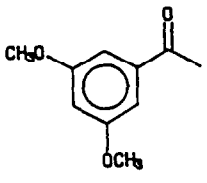
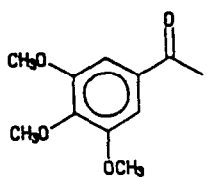
[2] E is the ratio of the specificity constants (k_{cat}/K_m) of the two enantiomers.[7]

However, we have found that the enantiospecificity can be markedly improved by structural changes in the aroylthio moiety of (±)-methyl-3-aroylthio-2-methylpropionate (Table 2). Especially noteworthy is the 3,5-dimethoxybenzoylthio derivative, which was attacked by the lipase of *Mucor meihei* (E = 28) with a high degree of stereospecificity. The following experimental procedure is presented to illustrate its operational simplicity and suitability for large scale use.

A suspension of crude *Mucor meihei* lipase (400 mg, Amano MAP-10) in 3.5 ml of 0.2 M phosphate buffer, pH 7.0, was centrifuged for 10 min at 1,000 x G. The supernatant was

TABLE 2. Enantiospecific Hydrolysis of (±)-Methyl-3-aroylthio-2-methylpropionate by *Mucor meihei* Lipase.



R^1	Extent of conversion (%)	Enantiomeric[8] excess (%) Ester	Acid	E
	37	26	43	3
	55	37	30	3
	50	41	42	4
	39	27	42	3
	13	6	40	2
	47	59	66	8
	47	60	68	10
	18	13	58	4

[1]To one ml of 0.2 M potassium phosphate buffer, pH 7.0, was added 1 mmol of substrate and 50 mg of *Mucor meihei* lipase Amano MAP-10 powder. The contents were incubated at 22°C for 44-96 h under gentle stirring.

removed and added to 1 g of (±)-methyl-3-3',5'-dimethoxybenzoylthio-2-methylpropionate. The resulting suspension was gently stirred with a magnetic stirrer for 72 h at 23°C. The reaction mixture was then diluted with 5 ml of 0.2 M phosphate buffer, pH 7.0, and the mixture was centrifuged for 5 min at 1,000 x G. After washing the precipitate with two 3-ml portions of 0.2 M phosphate buffer, pH 7.0, the water insoluble ester (480 mg), $[\alpha]_D^{23}$ = +27.8° (c, 2.95, $CHCl_3$), was collected. The supernatant and the washings were combined and acidified to pH 2.0 with 2N HCl. The precipitate was collected by filtration to yield 350 mg (-)-3-3',5'-dimethoxybenzoylthio-2S-methylpropionic acid, $[\alpha]_D^{23}$ = -36.7° (c, 2.54, $CHCl_3$; ee = 0.80); one crystallization from isooctane afforded a sample, $[\alpha]_D^{23}$ = -43.38° (c, 5.26, $CHCl_3$; ee = 0.93).

This strategy of enhancing biochemical stereospecificity by systematically altering structural features of bifunctional substrates extends the usefulness of enzymes and the concept could be of general applicability to biochemical catalyses.

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References and Notes

1. For part VII in this series, see: S. H. Wu, L. Q. Zhang, C. S. Chen, G. Girdaukas and C. J. Sih, Tet. Lett., 4323 (1985).
2. M. A. Ondetti, B. Rubin and D. W. Cushman, Science, 196, 441 (1977); M. A. Ondetti and D. W. Cushman, J. Med. Chem., 24, 355 (1981).
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6. M. Shimazaki, J. Hasegawa, K. Kan, K. Nomura, Y. Nose, H. Kondo, T. Ohashi and K. Watanabe, Chem. Pharm. Bull., 30, 3139 (1982).
7. The enantiomeric ratio (E value) is calculated from:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \qquad \text{where } c = ee_s/(ee_s + ee_p).$$

See: C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, J. Am. Chem. Soc., 104, 7294 (1982).
8. Enantiomeric excess (ee) was determined by comparison of the optical rotation with known standards and by PMR measurements of the methyl esters using $Eu(hfc)_3$.

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