Asymmetric Hydrolysis of ($\pm$)- α -Substituted Carboxylic Acid Esters with Microorganisms[†]

Shinobu IRIUCHIJIMA and Atsuko KEIYU

Sagami Chemical Research Center, Nishi-Ohnuma,
Sagamihara, Kanagawa 229, Japan

Received October 24, 1980

Microorganisms that hydrolyze methyl 2-phenylpropionate (**1**) or reduce 4-phenyl-2-butanone (**3**) were screened from 250 type cultures. Several *Aspergilli* and two bacteria hydrolyzed ester **1**, and *Asp. sojae* IAM 2703 preferentially hydrolyzed (*R*)-isomer of ($\pm$)-**1**, whereas *Bacillus subtilis* var. *niger* IFO 3108 and *Mycobacterium smegmatis* ATCC 10143 preferentially hydrolyzed (*S*)-isomer. The hydrolysis of the related esters of **1** with these organisms was also examined.

Esters containing an asymmetric center(s) at the alcohol part are asymmetrically hydrolyzed with microorganisms or microbial enzymes,¹⁾ but asymmetric hydrolyses of esters having an asymmetric center(s) at the acid part are scarcely known. There are many useful optically active acids or esters, e.g. mandelic acid, naproxen[2-(6-methoxy-2-naphthyl)propionic acid],²⁾ ketoprofen[2-(3-benzoylphenyl)propionic acid],³⁾ and fenvalerate[α -(4-chlorophenyl)isovaleric acid ester].⁴⁾ We thought that they might be obtained by biochemical asymmetric hydrolysis of the racemic esters, and tried to find enzymes or microorganisms that hydrolyze such esters. We selected methyl 2-phenylpropionate (**1**) as a model substrate and examined its hydrolysis with commercially available enzymes to know that microbial lipases, such as *Candida cylindracea* lipase MY and *Mucor* lipase MAP-10, hydrolyzed **1** weakly while expensive pig liver esterase hydrolyzed **1** strongly. Therefore we tried to screen microorganisms which hydrolyze **1** strongly.

As a screening program we tried to find microorganisms which reduce 4-phenyl-2-butanone (**3**) to 4-phenyl-2-butanol (**4**) together with those which hydrolyze ester **1** to 2-phenylpropionic acid (**2**) (Scheme 1), by add-

SCHEME 1.

ing both substrates **1** and **3** simultaneously into the cultured medium. From almost 250 species of microorganisms, including molds, yeasts, bacteria and actinomycetes, we found that several *Aspergilli* and two bacteria (Table I) hydrolyzed ester **1**, while several yeasts and one mold (Table II) reduced ketone **3**. Among them *Asp. sojae* IAM 2703 (*A. sojae*), *Bacillus subtilis* var. *niger* IFO 3108 (*B. subtilis*) and *Mycobacterium smegmatis* ATCC 10143 (*M. smegmatis*) were chosen as representative ester-hydrolyzing organisms, and further work was carried out with these organisms.

Further experiments with these organisms disclosed that *A. sojae* preferentially hydrolyzed (*R*)-isomer of ($\pm$)-**1** to produce (*R*)-(-)-acid **2** of 71% e.e.,⁵⁾ whereas *B. subtilis* and *M. smegmatis* preferentially hydrolyzed (*S*)-isomer to give (*S*)-(+)-acid **2** of 83% e.e. and 75% e.e.,⁵⁾ respectively. Methyl 2-phenylbutyrate (**5**) was also hydrolyzed with these organisms, and *M. smegmatis* preferentially hydrolyzed (*S*)-isomer of ($\pm$)-**5** to produce (*S*)-(+)-2-phenylbutyric acid of 52% e.e.⁵⁾ However, more sterically hindered methyl α -

[†] Asymmetric Hydrolysis of Esters by Biochemical Methods. Part I.

FIG. 1. Esters Examined for Hydrolysis with Microorganisms.

TABLE I. MICROORGANISMS THAT HYDROLYZED ESTER 1

<i>Asp. flavus</i>	IAM	2119
<i>Asp. oryzae</i>	OUT	5039
<i>Asp. parasiticus</i>	IFO	4301
<i>Asp. sojae</i>	IAM	2703
<i>Asp. sojae</i>	IAM	2666
<i>Asp. sojae</i>	IAM	2031
<i>Asp. tamarii</i>	IAM	2014
<i>Asp. tamarii</i>	IAM	2138
<i>Asp. tamarii</i>	IAM	2502
<i>Bacillus subtilis</i> var. <i>niger</i>	IFO	3108
<i>Mycobacterium smegmatis</i>	ATCC	10143

TABLE II. MICROORGANISMS THAT REDUCED KETONE 3

<i>Candida utilis</i>	IAM	4233
<i>Pichia miso</i>	IAM	4526
<i>Pichia miso</i>	IAM	4585
<i>Pichia miso</i>	IAM	4682
<i>Rhodotorula rubra</i>	ATCC	20258
<i>Rhodotorula rubra</i>	IFO	0901
<i>Geotrichum candidum</i>	ATCC	20141

(4-chlorophenyl)isovalerate (**8**) was not hydrolyzed at all with these organisms.

Methyl indan-1-carboxylate (**9**) was hydrolyzed with *A. sojae* and *M. smegmatis*, and *A. sojae* preferentially hydrolyzed (*R*)-isomer of ($\pm$)-**9** to produce (*R*)-(-)-indan-1-carboxylic acid of 55% e.e.⁶⁾ Analogs of ester **1**, methyl 2-(6-methoxy-2-naphthyl)propionate (**12**) and methyl 2-(3-benzoylphenyl)propionate (**13**),

TABLE III. HYDROLYSIS OF ESTERS WITH THREE MICROORGANISMS

Esters	<i>A. sojae</i>	<i>B. subtilis</i>	<i>M. smegmatis</i>
1	+++ <i>R</i>	+++ <i>S</i>	+++ <i>S</i>
5	++	+	++ <i>S</i>
6	++	—	++
7	+	—	++
8	—	—	—
9	++ <i>R</i>	—	+++
10	—	—	—
11	—	—	—
12	+ <i>R</i>	—	+—
13	+ <i>R</i>	+	+ <i>S</i>

+++ , most hydrolyzed; > ++ > + > — , not hydrolyzed.

were hydrolyzed a little with these organisms, and *A. sojae* preferentially hydrolyzed (*R*)-isomer of ($\pm$)-**12** to produce (*R*)-(-)-2-(6-methoxy-2-naphthyl)propionic acid of 89% e.e.²⁾ while *M. smegmatis* preferentially hydrolyzed (*S*)-isomer of ($\pm$)-**13** to give (*S*)-(+)-2-(3-benzoylphenyl)propionic acid of 38% e.e.³⁾ Hydrolysis of other esters together with those described above are summarized in Table III.

In this study microorganisms selected by hydrolyzing ester **1** hydrolyzed weakly the esters having a larger aromatic ring, such as **12** and **13**, while they could not hydrolyze esters having a more sterically hindered alkyl group or a hydroxyl group, such as **8**, **11** and **10**. Hydrolysis of esters having an asymmetric center at the α -position of the acid part is thought to be strongly affected by the nature of α -substitution, perhaps because enzymes attack the ester carbonyl carbon immediately next to the α -position. The result suggests that esters which have a relatively low molecular weight and are not so sterically hindered may be hydrolyzed by these organisms.

Further work on microorganisms which reduce ketone **3** will be reported in the future.

EXPERIMENTAL

Optical rotation was measured with an automatic digital polarimeter PM-201 (Union Giken Co.). Gas chro-

matography (GC) was performed with a Shimadzu GC-3BT gas chromatograph with a column of 15% EGTPA (2.1 m, 200°C).

Synthesis of substrates. Methyl 2-phenylpropionate was synthesized from commercially available 2-phenylpropanal by oxidation with Jones reagent followed by esterification with SOCl_2 -MeOH.⁷⁾ Methyl 2-phenylbutyrate and methyl α -(4-chlorophenyl)isovalerate were prepared from the corresponding arylacetonitriles by α -alkylation⁸⁾ followed by acid-hydrolysis and esterification. Methyl indan-1-carboxylate was obtained from indene and CO_2 ⁹⁾ followed by hydrogenation and esterification. Methyl 2-(6-methoxy-2-naphthyl)propionate was prepared from (*S*)-naproxen by esterification followed by racemization with NaOMe-MeOH, and methyl 2-(3-benzoylphenyl)propionate was synthesized from ketoprofen by esterification. 4-Phenyl-2-butanone, methyl 2-ethylhexanoate, butyl 2-ethylhexanoate, ethyl mandelate and ethyl chrysanthemate (*cis:trans*=30~40:60~70) were from commercial sources.

Screening of microorganisms which hydrolyze methyl 2-phenylpropionate (1) or reduce 4-phenyl-2-butanone (3) to 4-phenyl-2-butanol (4). Each microorganism was cultured by shaking in the appropriate medium (see below) (10 ml) contained in a test tube for 2~12 days at 30°C. Toluene (2 drops), ester 1 (1 drop) and ketone 3 (1 drop) were added to the culture, and the mixture was shaken overnight. To the mixture were added 6N H_2SO_4 (0.2 ml) and ethyl acetate (3 ml), and this was shaken for 5 min. The separated organic layer was injected into a gas chromatograph with a syringe to detect ester 1, acid 2, ketone 3 and alcohol 4.

For molds and yeasts, Y-medium (glucose 1%, peptone 0.5%, yeast extract 0.3%, malt extract 0.3%, pH 5~6) was used. For bacteria, B-medium (powdered bouillon 2%, yeast extract 0.5%, pH 7.2) or C-medium (glucose 1%, peptone 0.7%, yeast extract 0.5%, K_2HPO_4 0.5%, pH 7) was used. For actinomycetes, A-medium (glucose 1%, peptone 0.2%, meat extract 0.1%, yeast extract 0.1%, pH 7.2) was used.

Hydrolysis of ester 1 with *A. sojae*, *B. subtilis* and *M. smegmatis*. *A. sojae* was cultured by shaking in Y-medium (50 ml) in a 500-ml Erlenmeyer flask for 3 days. To the culture was added ester 1 (700 mg), and the hydrolysis was done by shaking overnight. The mixture which was acidified with 6N H_2SO_4 was shaken with ethyl acetate. The mixture was filtered to remove the insoluble matter. The filtered aqueous layer was extracted with ethyl acetate again, and the organic extracts were combined. GC analysis of the extract showed that 1 and 2 were in the ratio 4:6. It was shaken with 0.2M NaHCO_3 , and the aqueous layer was acidified and extracted with ethyl acetate. The extract was dried, concentrated and distilled to give (*R*)-acid 2 (107 mg) having $[\alpha]_D^{25} -65.8^\circ$ ($c=1$, benzene) (71% e.e.) [as compared with $[\alpha]_D^{25} -93.1^\circ$

($c=1.544$, benzene) (*R*)].⁵⁾

B. subtilis was cultured in two test tubes, each containing C-medium (10 ml) for 3 days. To the combined cultures was added ester 1 (200 mg), and the mixture was shaken overnight. The mixture was acidified and extracted with ethyl acetate. GC of the extract showed that 1 and 2 were in the ratio 7:3.

The acid fraction obtained by using NaHCO_3 solution produced (*S*)-2 (50 mg) having $[\alpha]_D^{25} +76.9^\circ$ ($c=0.5$, benzene) (83% e.e.).⁵⁾

M. smegmatis was cultured in three test tubes, each containing C-medium (10 ml) for 4 days. To the combined cultures was added 1 (240 mg), and the mixture was stirred overnight. The mixture was extracted as above. GC of the extract showed that 1 and 2 were in the ratio 6:4. The acid fraction gave (*S*)-2 (65 mg) having $[\alpha]_D^{25} +70.0^\circ$ ($c=0.65$, benzene) (75% e.e.).⁵⁾

Hydrolysis of methyl 2-phenylbutyrate (5) with *M. smegmatis*. To the culture (40 ml) was added ester 5 (135 mg), and the hydrolysis was done by shaking overnight. The mixture was extracted with ethyl acetate, and GC of the extract showed that ester 5 and its acid were in the ratio 75:25. The acid fraction gave (*S*)-2-phenylbutyric acid (24 mg) having $[\alpha]_D^{20} +50.0^\circ$ ($c=1.2$, benzene) (52% e.e.) [compared with $[\alpha]_D^{13-20} -96.8^\circ$ ($c=1.37$, benzene) (*R*)].⁵⁾

Hydrolysis of methyl indan-1-carboxylate (9) with *A. sojae*. *A. sojae* was cultured in Y-medium (100 ml) in a 500-ml Erlenmeyer flask for 4 days. The culture was filtered to give a mycelium which was pressed and dried at room temperature overnight, producing air-dried *A. sojae* (ca. 800 mg). The chopped air-dried *A. sojae* (180 mg) and ester 9 (200 mg) were stirred in water (20 ml) overnight. The mixture was extracted with ethyl acetate. GC of the extract showed that ester 9 and its acid were in the ratio 7:3. The acid fraction afforded (*R*)-indan-1-carboxylic acid (40 mg) having $[\alpha]_D^{20} +24.0^\circ$ ($c=0.4$, benzene) (56% e.e.) [compared with $[\alpha]_D^{25} +43.3^\circ$ ($c=2.59$, benzene) (*R*)].⁶⁾

Hydrolysis of methyl 2-(6-methoxy-2-naphthyl)propionate (12) with *A. sojae*. A mixture of air-dried *A. sojae* (400 mg), 12 (160 mg) and toluene (0.2 ml) in water (20 ml) was stirred overnight. The mixture was acidified and extracted with ethyl acetate. The acid fraction produced (*R*)-2-(6-methoxy-2-naphthyl)propionic acid (26 mg) having $[\alpha]_D^{25} -58.5^\circ$ ($c=1.3$, CHCl_3) (89% e.e.) [compared with $[\alpha]_D +66^\circ$ (CHCl_3) (*S*)].²⁾

Hydrolysis of methyl 2-(3-benzoylphenyl)propionate (13) with *M. smegmatis*. *M. smegmatis* was cultured in three test tubes, each containing C-medium (10 ml) for 4 days. To the combined cultures was added 13 (176 mg), and the mixture was stirred overnight. The mixture was acidified and extracted with ethyl acetate. The acid frac-

tion which contained 2-(3-benzoylphenyl)propionic acid (35 mg) having $[\alpha]_D^{23} + 20.5^\circ$ ($c = 1.17$, CH_2Cl_2) was chromatographed on silica gel with CH_2Cl_2 to yield (S)-acid (29 mg) having $[\alpha]_D^{23} + 21.7^\circ$ ($c = 1$, CH_2Cl_2) (38% e.e.) [compared with $[\alpha]_D^{23} + 57.1^\circ$ ($c = 0.76$, CH_2Cl_2) (S)].³⁾

REFERENCES

- 1) For example, W. J. Marsheck and M. Miyano, *Biochim. Biophys. Acta*, **316**, 363 (1973); T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **38**, 1965 (1974); Y. Yamaguchi, A. Komatsu and T. Moroe, *Nippon Nôgeikagaku Kaishi*, **50**, 619 (1976).
- 2) I. T. Harrison, B. Lewis, P. Nelson, W. Rooks, A. Roszkowski, A. Tomolonis and J. H. Fried, *J. Med. Chem.*, **13**, 203 (1970).
- 3) S. Rendic, V. Sunjic, F. Kajfez, N. Blazevic and T. Alevic-Kolbah, *Chimia*, **29**, 170 (1975); For other optically active anti-inflammatory agents, see T. Y. Shen, *Angew. Chem. Internat. Edit.*, **11**, 460 (1972).
- 4) M. Miyakado, N. Ohno, Y. Okuno, M. Hirano, K. Fujimoto and H. Yoshioka, *Agric. Biol. Chem.*, **39**, 267 (1975).
- 5) T. Fujita, K. Kawazu and T. Mitsui, *Agric. Biol. Chem.*, **30**, 1277 (1966).
- 6) A. Fredga, *Chem. Ber.*, **89**, 322 (1956); For a optically active anti-inflammatory agent of indan-1-carboxylic acid type, see S. Kuzuna, N. Matsumoto, T. Kometani and K. Kawai, *Japan J. Pharmacol.*, **24**, 695 (1974).
- 7) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).
- 8) M. Miyakado, N. Ohno, Y. Okuno, M. Hirazo, K. Fujimoto and H. Yoshioka, *Agric. Biol. Chem.*, **39**, 267 (1975).
- 9) N. Matsumura, Y. Yagyu and E. Imoto, *Nippon Kagaku Kaishi*, **1977**, 1344.