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MICROBIOLOGICAL KINETIC RESOLUTION AND ASYMMETRIC HYDROLYSIS OF 3,5-DIACETOXYCYCLOPENT-1-ENE

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Abstract—A 1:1 mixture of *cis*- and *trans*-3,5-diacetoxycyclopent-1-ene (1) was asymmetrically hydrolysed by baker's yeast to give *trans*-3(*R*)-acetoxy-5(*R*)-hydroxycyclopent-1-ene (*R*-**2a**) and S-predominant 3,5-dihydroxycyclopent-1-ene (3) accompanied by *trans*-3(*R*),5(*R*)-diacetoxycyclopent-1-ene (*R*-**1a**).

The optical activities of the products were found to be dependent on the difference of the enzymatic hydrolytic rate among *cis*-, *trans*(*S*,*S*)- and *trans*(*R*,*R*)-3,5-diacetoxycyclopent-1-ene.

The asymmetric hydrolysis was also investigated on wheat germ lipase, citrus acetyl esterase, and the lipase prepared from *Aspergillus niger*.

INTRODUCTION

Microbiological transformation is a conventional method to produce optically active compounds without using a resolution method or to introduce new asymmetric centers to compounds.

Recently, in the total synthesis of prostaglandins a variety of transformations with microorganisms have been utilized to overcome the stereochemical problems.

The microbial reduction[1] of *dl*-(6-carbomethoxyhexyl)cyclopentane-1,3,4-trione using the fungus of *Dipodascus uninucleatus* or *Mucor rammanianus* generated a new chiral center (*R* or *S*) in the molecule. This made possible the synthesis of naturally occurring prostaglandins. The microbiological hydrolysis[2] of 7-(2-*trans*-styryl-3-acetoxy-5-oxocyclopentene) heptanoic acid yielded an optically active alcohol and dispensed with the resolution method. The microbial oxidation[3] of 7-(5-oxocyclopentene) heptanoic acid to an important prostaglandin synthon introduced an asymmetric OH group into the molecule.

In order to develop a short synthesis of prostaglandins, a method using conjugated addition of an organometallic reagent to an enone system and subsequent alkylation of the directed enolate ion produced has recently been developed.[4-6] The synthesis of 11-deoxyprostaglandin has been achieved by application of this method. The synthesis of optically active protected 4-hydroxycyclopent-2-en-1-one would provide a possible synthesis of naturally occurring prostaglandins.[24]

There are many reports on the microbial[7-10] or enzymatic[11,12] hydrolyses and on the hydrolysis with β-cyclodextrin[13] as a model enzyme. Especially the enantioselective microbiological hydrolysis of *dl*-isopulegyl, *dl*-carvomethyl, *dl*-2- or -3-alkylcyclohexyl, *dl*-bornyl, and *dl*-1- ro -2-decalyl esters have been reported to give optically active alcohols. However, there is no evidence, to our knowledge, that a simple molecule with two equivalent ester functions such as 3,5-diacetoxycyclopent-1-ene (1) has been asymmetrically half-hydrolysed by microorganisms or enzymes.

As a part of our attempt to synthesise prostaglandins using bio-conversion methods, we tried an asymmetric hydrolysis of 3,5-diacetoxycyclopent-1-ene[14] **1** by microorganisms to *trans*-3(*R*)-acetoxy-5(*R*)-hydroxycyclopent-1-ene (*R*-**2a**) which could then easily be converted to 4(*R*)-hydroxycyclopent-2-en-1-one derivatives, important intermediates for the synthesis of prostaglandins as well as some other biologically active compounds. A detailed account of the conversion of *R*-**2a** to this key intermediate is given in the accompanying paper.[15]

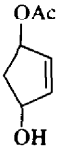
In the present paper, we describe the asymmetric hydrolysis of 3,5-diacetoxycyclopent-1-ene **1** by baker's yeast and some enzymes.

Structural identification and enantiomeric composition of the hydrolysed products

Incubation of *ca* 1:1 mixture of *cis*- and *trans*-3,5-diacetoxycyclopent-1-ene (**1**) with baker's yeast and chromatography of the product gave two hydrolysed compounds, *trans*-3(*R*)-acetoxy-5(*R*)-hydroxycyclopent-1-ene (*R*-**2a**) and 3,5-dihydroxycyclopent-1-ene (**3**), and *trans*-3(*R*),5(*R*)-diacetoxycyclopent-1-ene (*R*-**1a**) among other by-products.

1

R-**1a**: 3(*R*),5(*R*)
S-**1a**: 3(*S*),5(*S*)
1b: 3(*R*),5(*S*)



2

R-**2a**: 3(*R*),5(*R*)
S-**2a**: 3(*S*),5(*S*)
RS-**2b**: 3(*R*),5(*S*)
SR-**2b**: 3(*S*),5(*R*)

3

R-**3a**: 3(*R*),5(*R*)
S-**3a**: 3(*S*),5(*R*)
3b: 3(*R*),5(*S*)

Structural identification of these compounds is based on NMR, IR, mass spectral and VPC data.[15]

The hydrolysed product, *R*-**2a**, was found to have *trans* geometry by consideration of the methylene absorption pattern in the NMR spectrum. The compound *R*-**2a** exhibited two almost equivalent C-4 methylene protons at δ 2.12 and 2.22. Considering the fact that two equivalent C-4 methylene protons of *trans*-3,5-diacetoxycyclopent-1-ene **1a**[16] and *trans*-3,5-dihydroxycyclopent-1-ene **3a**[16]

appear at δ 2.18 and 1.78 in the NMR spectra, respectively, the compound *R*-**2a** was considered to be the *trans*-isomer. The *trans* geometry of *R*-**2a** was also confirmed by comparison with an authentic sample derived from *trans*-3,5-dihydroxycyclopent-1-ene **3a** by monoacylation. The isolated diacetate *R*-**1a** was also found to be the *trans*-isomer by its NMR spectrum. The diol **3**[16] was found to be a mixture of major *trans*-isomer **3a** and minor *cis*-isomer **3b** by gas chromatographic analysis.

As described in the accompanying paper,[15] the absolute configurations of these products were determined by the exciton chirality method[17] and the induced CD method.[18] Both *trans*-diacetate, *R*-**1a**, and *trans*-monoacetate, *R*-**2a**, had the 3(*R*),5(*R*)-configuration, while *trans*-diol **3a** had the 3(*S*),5(*S*)-configuration.

The enantiomeric composition of the hydrolysed product, *R*-**2a**, was determined by NMR measurement with a chiral shift reagent[18] (*vide infra*). The optical purity of the isolated diacetate, *R*-**1a**, was determined by conversion of monoacetate, *R*-**2a**, whose % e.e. was known, into the diacetate *R*-**1a** and by comparison of their specific rotations. The enantiomeric composition of the diol **3a** was also determined by a similar treatment (*vide infra*).

Hydrolysis with baker's yeast and some enzymes

Incubation of *ca.* 1:1 mixture of *cis*- and *trans*-3,5-diacetoxycyclopent-1-ene **1** with baker's yeast was carried out at 32° in the broth as described in the Experimental. The reaction was followed by sampling the mixture, extracting it with an organic solvent, and analysing the extract by gas-chromatography.

Variations of amounts of the substrates and the hydrolysed products in the mixture for the reaction time are shown in Fig. 1.

The substrate, *cis*-3,5-diacetoxycyclopent-1-ene **1b**, rapidly decreased and could hardly be detected after 15 hr. As the *cis*-isomer decreased, the hydrolysed product, *cis*-3-acetoxy-5-hydroxycyclopent-1-ene **2b**, rapidly increased to reach the maximum after *ca.* 3 hr, then rapidly decreased and could hardly be detected after 17 hr. On the other hand, the substrate, *trans*-3,5-diacetoxycyclopent-1-ene **1a**, began to decrease after the induction period of *ca.* 3 hr. The *trans*-monoacetate **2a** increased to reach a maximum after 12 hr and then it gradually decreased at approximately constant rate as the reaction proceeded.

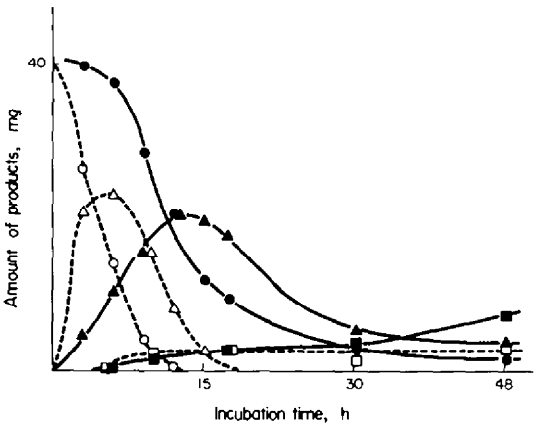


Fig. 1. Hydrolysis of *ca.* 1:1 mixture of 3,5-diacetoxycyclopent-1-ene **1** with baker's yeast: ●, *trans*-3,5-diacetoxycyclopent-1-ene **1a**; ▲, *trans*-3-acetoxy-5-hydroxycyclopent-1-ene **2a**; ■, *trans*-3,5-dihydroxycyclopent-1-ene **3a**; ○, *cis*-3,5-diacetoxycyclopent-1-ene **1b**; △, *cis*-3-acetoxy-5-hydroxycyclopent-1-ene **2b**; □, *cis*-3,5-dihydroxycyclopent-1-ene **3b**.

These observations indicate that the hydrolysis of the diacetate **1** with baker's yeast proceeded *via* the half-hydrolysed product **2** to give the completely hydrolysed product **3**.

The mixture was extracted with ethyl acetate to give the crude product which was chromatographed to afford three major products **1**, **2** and **3**.

Specific rotations and yields of these products for reaction time are summarized in Table 1.

Specific rotations of the diacetate **1** and the monoacetate **2** increased as the reaction proceeded, while that of the diol **3** decreased. These results indicate that the first hydrolysis of the diacetate **1** proceeded faster than the second one of the monoacetate **2**. Furthermore, hydrolyses of the diacetate **1** and the monoacetate **2** proceeded enantioselectively. In other words, a simultaneous kinetic resolution of the diacetate **1** and asymmetric synthesis of the monoacetate **2**, were effected by this hydrolysis.

In order to determine the enantiomeric composition of **1**, **2** and **3**, the NMR of (*dl*)-*trans*-3-acetoxy-5-hydroxycyclopent-1-ene **2a** obtained from (*dl*)-*trans*-diol

Table 1. Specific rotations and yields of products obtained by hydrolysis of 3,5-diacetoxycyclopent-1-ene **1** with baker's yeast

	3,5-Diacetoxy-cyclopent-1-ene **1**		3-Acetoxy-5-hydroxycyclopent-1-ene **2**		3,5-Dihydroxy-cyclopent-1-ene **3**	
Reaction time (h)	Yield (%)	$[\alpha]_D^{20}$	Yield (%)	$[\alpha]_D^{20}$	Yield (%)	$[\alpha]_D^{20}$
5	trans 16.8 cis 10.8	+8.3°	1.7 7.0	−17.1°	trace	
10	trans 14.9 cis 1.1	+45.1°	6.2 4.7	±0°	3.9 6.7	−24.2°
17	trans 9.1 cis 0	+185°	11.5 0	+143°	6.3 5.5	−44°
30	trans 2.5 cis 0	+199°	5.4 0	+199°	8.2 2.1	−37°
48	trans 0.6 cis 0	+215°	3.2 0	+229°	16.6 8.6	−14.5°

Yields were based on (*dl*)-*cis*- and *trans*-3,5-diacetoxycyclopent-1-ene.

3a by monoacylation exhibited a distinguishable pair of the C-5 proton signals due to *R* and *S* enantiometer by addition of NMR chiral shift reagent,[19] tris-(3-trifluoromethyl - hydroxymethylene - d - camphorato)-europium (III).

By the NMR measurement with the chiral shift reagent, the optically active *trans*-monoacetate **2a** ($[\alpha]_D^{20}$ + 229°) obtained by 48 hr incubation was shown to be composed of *R* (95%) and *S* (5%) enantiomer.

The maximum specific rotation of the *trans*-monoacetate **2a** was calculated to be +255° from the relationship between the specific rotation and the enantiomeric composition of **2a**.

The monoacetate **2a** ($[\alpha]_D^{20}$ + 229°, 90% e.e.) was acylated with acetic anhydride in pyridine at room temperature to give the diacetate **1a** ($[\alpha]_D^{20}$ + 208°). The maximum specific rotation ($[\alpha]_D$ max) for the pure **1a** was estimated to be +231°. Similar acylation of the diol **3** ($[\alpha]_D^{20}$ −81° **3a**; 66%, **3b**; 34%) gave the diacetate **1** ($[\alpha]_D^{20}$ − 73°, **1a**; 66%, **1b**; 34%). As the *cis*-diacetate **1b** is optically inactive, $[\alpha]_D$ max for the *trans*-diol **3a** was figured out to be −237°.†

Based on $[\alpha]_D$ max of **1a**, **2a**, and **3a**, optical purity‡ of each product was calculated and summarized in Table 2.

In the accompanying paper,[15] the absolute configuration of (+)-**2a** was determined to be the *R*-configuration. Hence (+)-**1a** derived from (+)-**2a** was the *R*-enantiomer, and (−)-**3a** which gave (−)-**1a** was the *S*-enantiomer.

This hydrolysis reaction is presumably successive reaction of the first order. An enantiomer, *trans*-3(*S*)-5(*S*)-diacetoxycyclopent-1-ene, *S*-**1a**, was hydrolysed faster than the *R*-enantiomer, *R*-**1a** to *trans*-3(*S*)-acetoxy-5(*S*)-hydroxycyclopent-1-ene, *S*-**2a**, which was also hydrolysed successively faster than the *R*-enantiomer, *R*-**2a**, to give *trans*-3(*S*),5(*S*)-dihydroxycyclopent-1-ene, *S*-**3a**.

Therefore, the optical activities of the products obtained by the hydrolysis with baker's yeast depended on (1) the difference of the hydrolysis rate between *cis*-, *trans*-(*S*,*S*)- and *trans*-(*R*,*R*)-3,5-diacetoxycyclopent-1-ene and (2) the difference in the hydrolysis rate between the diacetate **1** and the monoacetate **2**. Thus, these differences in the hydrolysis rate between geometrical and enantiomeric isomers resulted in a net accumulation of the *trans*-(*R*)-monacetate **2a** and recovery of the kinetically resolved *trans*-(*R*)-diacetate **1a**.

From the viewpoint of stereoselectivity in the enzymatic hydrolysis of symmetrical 3,5-diacetoxycyclopent-1-ene having two asymmetric centers, it is interesting to note that the enzyme or enzymes in baker's yeast had both geometrical selectivity on the *cis*-, *trans*-isomer of the cyclopentene moiety and enantiomeric selectivity. It is, however, obscure whether the first hydrolysis of the diacetate **1** to the monoacetate **2** and the second hydrolysis of **2** to the diol **3** were catalysed by the same enzyme or different enzymes in baker's yeast. Baker's yeast has been previously known to contain several enzymes which have different catalytic function such as hydrolysis,[2,8] reduction[22] and dehydrogenation.[23] Among the byproducts, 1-acetoxy-3-hydroxycyclopentane was isolated and identified.

It was considered that the rapid decrease of *cis*-3,5-diacetoxycyclopent-1-ene or *cis*-3-acetoxy-5-hydroxycyclopent-1-ene during the hydrolysis could to a certain extent be attributed to the formation of *cis*-3-acetoxy-5-hydroxycyclopentane by the reductase of baker's yeast, although the *cis*-compounds would be assimilated by the cells of baker's yeast. In fact, the isolated, 3-acetoxy-5-hydroxycyclopentane was found to be the *cis*-isomer by comparison of its retention time on GLC with an authentic sample prepared by hydrogenation of *cis*-3-acetoxy-5-hydroxycyclopent-1-ene.

When the diacetate **1** was incubated with enzymes such as wheat germ lipase, citrus acetyl esterase,[20] and the lipase[21] prepared from *Aspergillus niger*, 3-acetoxy-5-hydroxycyclopent-1-ene **2** was obtained recovering the substrate, but 3,5-dihydroxycyclopent-1-ene **3** was not detected. The results are shown in Table 3.

In all cases the hydrolysed product **2** was found to be a mixture of *cis*- and *trans*-isomer which had a smaller specific rotation in comparison with the product from baker's yeast.

In conclusion, we obtained the optically active *trans*-3(R),5(R)-diacetoxycyclopent-1-ene, *R*-**1a**, *trans*-3(*R*)-aceotxy-5(*R*)-hydroxycyclopent-1-ene, *R*-**2a**, and *S* predominant 3,5-dihydroxycyclopent-1-ene **3** from a 1:1 mixture of *cis*- and *trans*-3,5-diacetoxycyclopent-1-ene **1** by hydrolysis with baker's yeast. All the optically active compounds *R*-**1a**, *R*-**2a**, and *S*-**3** are convertible to an important chiral prostaglandin synthon as described in the accompanying paper.[15]

EXPERIMENTAL

As overhead stirrer Yamato Labo. Stirrer L-35 was used. IR spectra were recorded on Hitachi EPI-510 spectrometer. NMR spectra were taken on a Varian EM 360 (60 MHz), a JEOL JNM-MH-100 (100 MHz), and a JEOL-PS-100 (100 MHz) spectrometer. The chemical shifts (δ) were given on the basis of TMS as the internal standard. Mass spectra were determined on a LKB 9000 mass spectrometer, at 70 eV. All optical rotations were measured in MeH on JASCO J-20 automatic recording spectropolarimeter. Layer chromatography was performed using Merk silica gel (Kiesel gel 60 F254). GLC was carried out with Hitachi 073 gas

†This value is the average one.

‡The enantiomeric composition of **2a** was also confirmed[15] by NMR measurement of the diastereomeric α-methyl-α-trifluoromethylphenylacetate of 4-hydroxycyclopent-2-en-1-one derived from **2a**.

Table 2. Optical purities of the products by the hydrolysis with baker's yeast

	Optical purity (%)		
Incubation time (h)	3,5-Diacetoxy-cyclopent-1-ene **1**	3-Acetoxy-5-hyd-roxycyclopent-1-ene **2**	3,5-Dihydroxy-cyclopent-1-ene **3**
17	80	56	32
30	86	78	19
48	93	90	10

Table 3. Hydorolysis of 3,5-diacetoxycyclopent-1-ene 1 with some enzymes

Enzymes	Incubation time (h)	3,5-Diacetoxy-cyclopent-1-ene 1 Yield (%)	3,5-Diacetoxy-cyclopent-1-ene 1 $[\alpha]_D^{20}$	3-Acetoxy-5-hydroxycyclopent-1-ene 2 Yield (%)	3-Acetoxy-5-hydroxycyclopent-1-ene 2 $[\alpha]_D^{20}$
Wheat germ. lipase	24	20.0	+48.6°	32.0	+3.0°
Citrus acetyl esterase	24	50.0	−7.4°	39.0	+7.7°
Lipase prepared from *Aspergillus niger*	48	52.4	−29.9°	13.5	+107°

chromatography using a column (3 mm × 2 m) packed with 20% carbowax 20 M on chromosorb W (NAW) at 180°C.

Organism. Baker's yeast used was a commercial brand of baker's yeast produced Oriental Yeast Co.

Enzymes

Wheat germ lipase (glycerol-ester hydolase EC. NO. 3.1.1.3.) was purchased from Sigma chemical Co.

Citrus acetyl esterase was prepared as follows. The outside skin was removed from 40 Japanese mandarins and weighed-680 g. After the cutting to small pieces and the addition of 1.8 l. of 0.2% NaCl aq, the outside skin was homogenised with the homogeniser (Universal homogenizer, Nihon Seiki). The homogenate was filtered through the gauze and further centrifuged at 5000 rpm at 4° for 10 min, and the supernatant fluid was collected. Ammonium sulfate was added to give a final saturation of 70%, which was allowed to stand overnight at 4°. The precipitated protein was harvested by centrifugation at 8000 rpm at 4° for 15 min. The pellet was dissolved in a small volume of deionised water. The fraction was dialysed against tap water and then deionised water. The dialysed fraction was centrifuged at 8000 rpm 4° for 15 min and the residue removed. The clear supernatant soln was designed "crude citrus acetyl esterase".

Lipase was prepared from Asperigillus niger *as follows.* Cells of *Asperigillus niger* ATCC 9142 were grown in a medium containing (per liter): technical glucose, 20 g; monopotassium phosphate, 1.5 g; magnesium sulfate heptahydrate, 1.5 g; ammonium nitrate, 1.0 g; enzyme hydrolysed lactoalbumin, 1.0 g; corn steep liquor solids, 1.0 g; soluble component of autolysed yeast, 0.5 g; L-glutamic acid, 0.5 g; zinc sulfate heptahydrate, 10 mg. The broth fermented at 28° for 48 hr under shaking with rotary shaker was sonicated at 4° for 15 min. The sonicated soln was centrifuged at 4° at 8000 rpm for 20 min and the clear supernatant fluid was collected. After adjusting to pH 5.7 with 30% NaOH aq, ammonium sulfate was added to give a final saturation of 45%, which was allowed to stand overnight at 4°. The ppt was removed by the centrifugation at 8000 rpm for 20 min. Further, ammonium sulfate was added to give a final saturation of 60%, which was allowed to stand overnight at 4°. The precipitated protein was harvested by centrifugation at 8000 rpm for 20 min. The pellet was dissolved in a small volume of deionised water and then 0.05 M acetate buffer (pH 5.6). The clear soln was designed "crude lipase from A. niger".

Substrate. 3,5-Diacetoxycyclopent-1-ene was prepared by our method.[14] A mixture of 54:46 ratio of *cis*- and *trans*-**1** was used as the substrate in the hydrolysis reaction with baker's yeast and enzymes as described above.

Incubation of a mixture of cis- *and* trans-3,5-*diacetoxycyclopent-1-ene* **1** *with baker's yeast*

(a) *Incubation for* 48 *hr.* Baker's yeast cells (270 g; compressed cake) were added to 0.9 l. deionised water. In a second 0.9 l portion of deionised water, 90 g of glucose and 67.5 g of NaH_2PO_4 were dissolved. The yeast-cells suspension was then mixed with the glucose soln and allowed to stand at room temp for 2 hr. To this suspension 18 g of a mixture of *cis*- and *trans*- **1** was added. The mixture was vigorously agitated at 32° by overhead stirrer in a 5.0 l. separable round-bottomed flask. Incubation was terminated after 48 hr. The incubated suspension was centrifuged at 4000 rpm for 10 min to separate into clear supernatant and yeast cells. The supernatant was saturated with ammonium sulfate and exhaustively extracted 5 times with EtOAc. The separated yeast cells were triturated and extracted with EtOAc. The extracted EtOAc portions were combined and dried over Na_2SO_4. Removal of EtOAc *in vacuo* afforded 6.93 g of a crude product which mainly exhibited four spots (R_f 0.54, 0.24, 0.20 and 0.03) by TLC (EtOAc–benzene, 1:1). This oily crude product was chromatographed using a column (49 × 2 cm) packed with silica gel (Wakogel C-200). The column was eluted with a gradient system consisting of 1.0 l of benzene in the mixing chamber and 1.0 l. of EtOAc in the reservoir, and 8 ml fractions were collected.

(i) Fractions 23–35 were pooled and concentrated to afford 110 mg of *R*-**1a** (yield; 0.6%), which was homogenous by TLC (R_f 0.54; EtOAc–benzene, 1:1) and by GLC (retention time: 12.3 min); $[\alpha]_D^{20}$ +215° (*c*, 0.023, MeOH); IR (liquid): 1735, 1240 and 1035 cm^{-1}; NMR (60 MHz, CCl_4): 2.00 (6H, s, $OCOCH_3$), 2.21 (2H, t, J = 6 Hz, H-C_4), 5.73 (2H, t, J = 6 Hz, H-C_1 & H-C_3), 6.06 (2H, s, H-C_1 & H-C_2); mass spectrum (70 eV; *m/e*, %): 141 (M–$COCH_3$, 2), 125 (73), 124 (65), 99 (27), 82 (100), 43 (80). These data were identical with those of (*dl*)-*trans*-3,5-diacetoxycyclopent-1-ene, reported in the literature.[16]

(ii) Fractions 56–75 were pooled and concentrated to afford 450 mg of *R*-**2a** (yield; 3.2%), which was homogenous by TLC (R_f 0.24; EtOAc–benzene, 1:1) and by GLC (retention time: 21.7 min); $[\alpha]_D^{20}$ +229° (*c*, 0.040, MeOH); IR (liquid): 3350, 1720, 1055 and 1020 cm^{-1}; NMR (100 MHz, $CDCl_3$): 1.90 (1H, broad s, OH), 2.02 (3H, s, $OCOCH_3$), 2.12 (1H, dd, J = 5 Hz & 8 Hz, H-C_4), 2.22 (1H, dd, J = 5 Hz & 8 Hz, H-C_4), 5.08 (1H, m, H-C_5), 5.84 (1H, m, H-C_3), 6.04 & 6.16 (2 H, 2 dm, J = 7 Hz, H-C_1 & H-C_2); mass spectrum (70 eV; *m/e*, %): 99 (M–$COCH_3$, 3), 82 (100), 43 (80).

(iii) Fractions 190–214 were pooled and concentrated to afford 2.47 g of **3** (yield; 25.2%), which was homogenous by TLC (R_f 0.03; EtOAc–benzene, 1:1) and was a mixture of 34:66 ratio of *cis*- and *trans*-isomers by GLC (retention time: 20.8 min, *cis*-isomer; 22.5 min, *trans*-isomer); $[\alpha]_D^{20}$ -14.5° (*c*, 0.127, MeOH). This compound was identical (IR, NMR and mass spectrum) with an authentic one.[16]

(iv) Fractions 86–95 were pooled and concentrated to afford 108 mg of *cis*-1-acetoxy-3-hydroxycyclopentane, which was homogenous by TLC (R_f 0.20; EtOAc–benzene, 1:1) and by GLC (retention time: 15.3 min), and was identical (retention time on GLC) with an authentic (*dl*)-*cis*-1-acetoxy-3-hydroxycyclopentane (*vide infra*); $[\alpha]_D^{20}$ +18.9° (*c*, 0.040, MeOH); IR (Liquid): 3350, 1720, 1240, 1050 and 1020 cm^{-1}; NMR (60 MHz, CCl_4): 1.62 & 2.24 (2H, m, H-C_2), 1.80 (4H, m, H-C_4 & H-C_5), 2.00 (3H, s, $OCOCH_3$), 4.03 (1H, broad s, OH), 4.18 (1H, m, H-C_3), 4.95 (1H, m, H-C_1); mass spectrum (70 eV; *m/e*, %): 101 (M–$COCH_3$, 2), 84 (65), 61 (23), 55 (30), 43 (100).

(b) *Incubation for* 5 *hr,* 10 *hr,* 17 *hr and* 30 *hr.* Results are summarized in Table 1.

GLC quantitative analysis of reaction mixture of the incubation with baker's yeast

On the incubation of the diacetate **1** (20 g) as described above, 10 ml of aliquot was sampled every 3 hr from the mixture.

Tetralin (25 μl) as the internal standard was added to the sample and the mixture was saturated with NaCl and extracted with ether (1 × 10 ml). The organic layer was analysed by GLC to calculate amounts of each component in the mixture based on the internal standard tetralin. The results are shown in Fig. 1.

Incubation of **1** *with enzymes*

(a) *With wheat germ lipase for 24 hr.* Wheat germ lipase (500 mg) was dissolved in 1.0 l. of 0.05 M acetate buffer (pH 5.5) and then 3.0 g of the diacetate **1** was added to the enzyme soln. The mixture was vigorously sitrred by overhead stirrer in 2.0 l. round-bottomed flask keeping the temp at 32°. Incubation was terminated after 24 hr. The mixture was thoroughly extracted 5 times with EtOAc.

The extracts were dried (Na_2SO_4) and evaporated to afford 1.35 g of a crude product, which exhibited two spots (R_f 0.54 and R_f 0.24) by TLC (EtOAc–benzene, 1:1). The crude product was chromatographed on preparative TLC with EtOAc–benzene (1:1) to give 540 mg (20.0%) of **1** and 710 mg (32%) of **2**.

1; ratio of *cis*- and *trans*-isomer; 70:30 $[\alpha]_D^{20}$ +48.6° (*c*, 0.060, MeOH).

2; ratio of *cis*- and *trans*-isomer; 52:48 $[\alpha]_D^{20}$ +3.0° (*c*, 0.098, MeOH).

These products were identical (IR, NMR and mass spectrum) with the authentic ones.

(b) *With citrus acetyl esterase for 24 hr.* "Crude citrus acetyl esterase (20 ml)" and 180 ml of 0.05 M phosphate buffer (pH 7.0) were mixed and then 3.0 g of the diacetate **1** was added to the enzyme soln. The mixture was vigorously stirred at 32° in 1.0 l. erlenmeyer flask.

Incubation was terminated after 24 hr. The mixture was treated as above to afford 2.97 g of a crude product which exhibited two spots (R_f 0.54 and R_f 0.24) by TLC (EtOAc–benzene, 1:1). This oily crude product was applied to a column (22 × 2 cm) packed with silica gel (Wakogel C-200). The column was eluted with a gradient system (EtOAc–benzene, 500 ml: 500 ml; one fraction volume: 12.0 ml).

(i) Fractions 1–13 were pooled and concentrated to afford 1.495 g (50.0%) of **1**, which was homogenous by TLC (R_f 0.54; EtOAc–benzene, 1:1) and was a mixture of 54:46 ratio of *cis*- and *trans*-isomer by GLC; $[\alpha]_D^{20}$ −7.38° (*c*, 0.058, MeOH).

(ii) Fractions 18–31 were pooled and concentrated to afford 909 mg (39.0%) of **2**, which was homogenous by TLC (R_f 0.24; EtOAc–benzene; 1:1) and was a mixture of 56:44 ratio of *cis*- and *trans*-isomer by GLC; $[\alpha]_D^{20}$ +7.7° (*c*, 0.203, MeOH). These products were identical (IR, NMR and mass spectrum) with the authentic samples.

(c) *With lipase prepared from* A. niger *for 48 hr.* Crude lipase from *A. niger* (75 ml) and 235 ml of 0.05 M acetate buffer (pH 5.3) were mixed and then 3.0 g of the diacetate **1** was added to the enzyme soln.

By the incubation under the same conditions and work-up as described, products were obtained as follows.

(i) 3,5-Diacetoxycyclopent-1-ene **1**; 1.54 g (52.4%) ratio of *cis*- and *trans*-isomer; 62:38. $[\alpha]_D^{20}$ −29.9° (*c*, 0.328, MeOH).

(ii) 3-Acetoxy-5-hydroxycyclopent-1-ene **2**; 306 mg (13.5%) ratio of *cis*- and *trans*-isomer; 47:53. $[\alpha]_D^{20}$ +107° (*c*, 0.102, MeOH).

(dl)-trans-3-*Acetoxy*-5-*hydroxycyclopent*-1-*ene*

A soln of acetyl chloride (696.5 mg, 9.0 mmol) in THF (6.0 ml) was added over 15 hr with a moter syringe drive at room temp to a stirred soln of (*dl*)-*cis*- and *trans*- **3** (700 mg, 7.0 mmol) and pyridine (830 mg, 10.5 mmol) in THF (20 ml).

The mixture was poured into *ca.* 150 ml of water and extracted with EtOAc (3 × 30 ml). The combined organic layer was washed with dil HCl, Na_2CO_3 aq, and NaCl aq, and then dried (Na_2SO_4).

Removal of the solvent gave a crude product (1.00 g), which was purified by preparative TLC (n-hexene–EtOAc, 1:1) to afford (*dl*)-cis- and *trans*- **2**, (413 mg, 29 mmol).

The *trans*- and *cis*-isomer (**2a** and **2b**) were separated by preparative GLC to yield **2b** (50 mg, 0.35 mmol) and **2a** (49 mg, 0.34 mmol). **2b**; IR (liquid): 3350, 1725, 1255, 1060 and 1020 cm^{-1}; NMR (100 MHz, $CDCl_3$): 1.62 (1H, dt, J = 4 Hz, & 14 Hz, H–C_4), 2.80 (1H, dt, J = 7 Hz & 14 Hz, H–C_4), 2.04 (3H, s, $OCOCH_3$), 2.70 (1H, broad s, OH), 4.73 (1H, m, H–C_5), 5.50 (1H, m, H–C_3), 5.96 & 6.12 (2H, 2 dm, J = 5 Hz, H–C_1 & H–C_2); mass spectrum (70 eV; *m/e*, %): 125 (M–OH, 25), 100 (50), 99 (85), 82 (100), 43 (85).

2a was identical (IR, NMR and mass spectrum) with *R*-**2a** obtained by baker's yeast.

(dl)-cis-*Acetoxy*-3-*hydroxycyclopentane*

A soln of **2a** (11 mg, 0.077 mmol) in MeOH (10 ml) was hydrogenated over 5% Pd–C (50 mg) under atmospheric pressure. The hydrogenation was complete in almost 40 min. The catalyst was filtered off and the filter cake was washed with MeOH. The evaporation of the solvent furnished 6 mg (0.042 mmol, 53%) of (*dl*)-*cis*-1-acetoxy-3-hydroxycyclopentane which was identical (retention time on GLC: 15.3 min) with 1-acetoxy-3-hydroxycyclopentane obtained as a by-product by hydrolysis of the diacetate **1** with baker's yeast.

Trans-3(R),5(R)-*Diacetoxycyclopent*-1-*ene* (R-**1a**) *from* trans-3(*R*)-*acetoxy*-5(R)-*hydroxycyclopent*-1-*ene* (R-**2a**)

Ac_2O (0.2 ml, 2 mmol) was added to a soln of *R*-**1a** (27.5 mg, 0.19 mmol. $[\alpha]_D^{20}$ +229° 90% R e.e.) in 0.3 ml of pyridine.

After stirring at room temp for 15 hr, ether (50 ml) and water (10 ml) were added and the separated organic layer were washed with dil HCl (3 × 10 ml), sat $NaHCO_3$ aq (3 × 10 ml) and brine, and then dried ($MgSO_4$). Removal of the solvent gave a colorless oil (36 mg). which was purified by preparative TLC (benzene–ether, 9:1) to afford *R*-**1a** (27.6 mg, 77%), $[\alpha]_D^{20}$ +208° (*c*, 0.025, MeOH).

3,5-*Diacetoxycyclopent*-1-*ene* (**1**) *from* 3,5-*dihydroxycyclopent*-1-*ene* (**3**)

Ac_2O (400 mg, 4 mmol) was added to a soln of **3** (92 mg, 0.92 mmol, *cis*:*trans* = 34:66, $[\alpha]_D^{20}$ −81° on the basis of *trans*-**3a**) in pyridine (0.5 ml).

After stirring at room temp for 15 hr ether (50 ml) and water (13 ml) were added and the separated organic layer was washed with dil HCl (3 × 10 ml), sat $NaHCO_3$ aq (3 × 10 ml) and brine, and then dried ($MgSO_4$). Evaporation of the solvent gave an oil (161 mg) which was purified by preparative TLC (benzene–ether, 9:1) to afford **1** (120 mg, 71%, *cis*:*trans* = 34:66), $[\alpha]_D^{20}$ −73° (MeOH) on the basis of trans-**1a**.

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REFERENCES

[1]C. J. Sih, J. B. Heather, G. P. Peruzzotti, P. Price, R. Sood and L. F. H. Lee, *J. Am. Chem. Soc.* **95**, 1667 (1973).
[2]W. J. Marsheck and M. Miyano, *Biochim. Biophys. Acta* **316**, 363 (1973).
[3]S. Kurozumi, T. Toru and S. Ishimoto, *Tetrahedron Letters* 4959 (1973).
[4]G. H. Posner, C. E. Whittern J. J. Sterling and D. J. Brunelle, *Ibid.* 2591 (1974).
[5]G. H. Posner, J. J. Sterling, C. E. Whitten, C. M. Lentz and D. H. Brunelle, *J. Am. Chem. Soc.* **97**, 107 (1975).
[6]J. W. Patterson, Jr. and J. H. Fried, *J. Org. Chem.* **39**, 2506 (1974).
[7a]T. Oritani and K. Yamashita, *Agr. Biol. Chem.* **37**, 1691 (1973); [b]T. Oritani and K. Yamashita, *Ibid.* **37**, 1691 (1973); [c]T. Oritani and K. Yamashita, *Ibid.* **37**, 1695 (1973); [d]T. Oritani and K. Yamashita, *Ibid.* **38**, 1691 (1974); [e]T. Oritani and K. Yamashita, *Ibid.* **38**, 1695 (1974); [f]T. Oritani and K. Yamashita, *Ibid.* **39**, 89 (1975).
[8]C. J. Sih, R. G. Salomon, P. Price, G. Peruzzotti and R. Sood, *Chem. Commun.* 240 (1972).

[9]T. Moroe, T. Hattori, A. Komatsu and Y. Yamaguchi, *Japan Pat.* 72-22276.
[10]T. Tosa, T. Mori, N. Fuse and I. Chibata, *Biotech. Bioeng.* **9**, 603 (1967).
[11]A. F. Kluge, K. G. Untch and J. H. Fried, *J. Am. Chem. Soc.* **94**, 7827 (1972).
[12]E. P. Abraham and G. G. Frederic, *U.S. Pat.* **3**, 202, 656.
[13]L. Vander Jagt, F. L. Killian and M. L. Bender, *J. Am. Chem. Soc.* **92**, 1016 (1970).
[14]T. Toru, S. Kurozumi, T. Tanaka, S. Miura, M. Kobayashi and S. Ishimoto, *Synthesis* 865 (1974).
[15]T. Tanaka, S. Kurozumi, T. Toru, S. Miura, M. Kobayashi and S. Ishimoto, *Tetrahedron* to be published (1976).
[16]F. G. Cocu, G. Wolczunowicz, L. Bors and T. Posternak, *Helv. Chim. Acta* **53**, 739 (1970).
[17]J. Dillon and K. Nakanishi, *J. Am. Chem. Soc.* **94**, 6190 (1972).
[18a]S. F. Mason, *J. Chem. Soc.* **B**, 370 (1966); [b]N. Harada and Nakanishi, *J. Am. Chem. Soc.* **91**, 3989 (1969); [c]N. Harada and K. Nakanishi, *Accounts Chem. Res.* **5**, 257 (1972); [d]M. Koreesa, N. Harada and K. Nakanishi, *J. Am. Chem. Soc.* **95**, 266 (1974).
[19a]C. C. Hinckley, *Ibid.* **91**, 5160 (1969); [b]G. M. Whitesides and D. W. Lewis, *Ibid.* **92**, 6979 (1970).
[20]E. F. Jansen, R. Jang and L. R. MacDonell, *Archiv. Biochem.* **15**, 415 (1947).
[21]J. Fukumoto, M. Imai and Y. Tsujisaka, *J. Gen. Appl. Microbiol.* **9**, 353 (1963).
[22]R. P. Lanzilotta, D. G. Brodley and K. M. McDonald, *Ibid.* **27**, 130 (1974).
[23]M. Akhtar and M. A. Parvez, *Biochem. J.* **108**, 527 (1968).
[24]Recently Stork *et al.* reported a total synthesis of prostaglandins using optically inaceive protected 4-hydroxycyclopent-2-en-1-one; G. Stork and M. Isobe, *J. Am. Chem. Soc.* **97**, 6260 (1975).