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Microbial asymmetric syntheses of 3-alkylphthalide derivatives

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Abstract: Phthalide derivatives, almost all of which have an *S*-configuration, have a wide range of activity and exist in *Angerica sinensis* Diels and *Sligusticum wallichiii* Franch. For the first time, optically active (*S*)-3-methylphthalide derivatives were synthesized using two methods, asymmetric microbial reduction and microbial hydroxylation. For the first method, methyl 2-acetylbenzoate was synthesized as a substrate, which was reduced asymmetrically by *Geotrichum candidum* IFO 34614 to obtain (*S*)-3-methylphtalide in 92% yield (99% enantiomeric excess, ee). For the second method, 2-ethylbenzoic acid was employed as a substrate which was hydroxylated asymmetrically at the benzylic position by either *Pseudomonas putida* ATCC 12633 or *Aspergillus niger* IFO 6661, whose fermentation was induced by *o*-toluic acid, to obtain (*S*)-3-methylphthalide in 80% yield (99% ee). (*S*)-3-Butylphthalide and (*S*)-3-octylphthalide were obtained in the same manner in 12% yield (ee=99%) and 10% yield (ee=99%), respectively. © 1997 Elsevier Science Ltd

Introduction

Phthalide derivatives which are present in the crude drug of *Angerica sinensis* Diels and *Sligusticum wallichii* Franch[1a,b] have various pharmacological effects such as: a relaxant effect on uterine[2] and teniae coli smooth muscles[3] and centrally acting muscle;[4] an antiproliferative effect on mouse aorta smooth muscle cells;[5] an antiplatelet effect;[6] a skin penetration enhancer;[7] and a vasodilation effect in rats.[8] For example, (*S*)-3-butylphthalide inhibits prostaglandin F_{2a}.[9] 3-Octylphthalide antagonizes Ca^{2+} and might decrease the Ca^{2+}-sensitivity of contractile elements or inhibit contractile proteins in the rat aorta.[8] Almost all of the phthalide derivatives which have biological functions have an *S*-configured chiral center.[10] It is necessary to synthesize stereogenic phthalide derivatives asymmetrically for their use as various pharmacological compounds. Ogawa *et al.* have reported that (*S*)-3-methylphthalide was synthesized in 83% ee using (*S*)-BINAL-H as its catalyst,[11] and Noyori *et al.* have synthesized a 97% ee of (*S*)-3-methylphthalide by the asymmetric reduction of ethyl 2-acetylbenzoate using (*S*)-BINAP-Ru(II) catalyst.[12] An optically active phthalide was also synthesized in adequate yield using (*S*)-2-methoxymethylpyrrolidine as a chiral auxiliary,[13] but this method was not efficient because of the many steps for its synthesis. A metallic catalyst is expensive and is difficult to dispose. We examined the synthesis of optically active 3-alkylphthalides by asymmetric reduction using a biocatalyst which was cheap, friendly to the environment, and efficient and by asymmetric oxidation which has not been performed before. Optically active benzyl alcohols with carboxylic acids at the *o*-position are used as precursors for optically active 3-alkylphthalides. The efficient synthesis of asymmetric benzyl alcohols is necessary to synthesize asymmetric phthalides. The retrosynthetic analysis of the asymmteric phthalide derivatives is shown in Scheme 1. It is possible to synthesize the precursors using biocatalysts which are derived from: (i) the asymmetric reduction of 2-acylbenzoic acids; (ii) the asymmetric hydroxylation of 2-alkylbenzoic acids; and (iii) the optical resolution of the racemic precursors using lipase catalysts.

Although the asymmetric reduction of acetophenone using a biocatalyst has been studied extensively,[14a–c] the asymmetric reduction of acetophenone substituents containing carboxylic acids at

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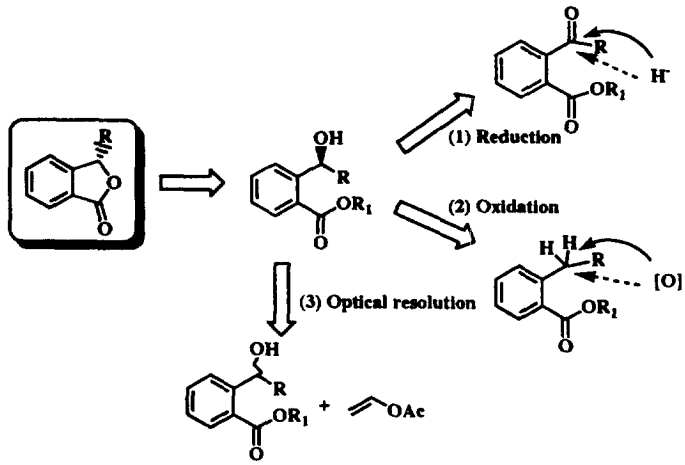


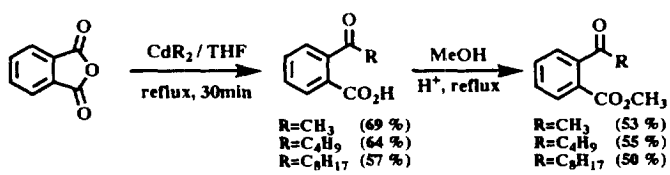
Scheme 1.

the *o*-position have not been studied [(1) in Scheme 1]. The carboxylic acid may inhibit the asymmetric reduction produced by the biocatalyst. Although asymmetric hydroxylation by microbes have been reported,[15a,b] the reaction at the benzylic position has not been characterized [(2) in Scheme 1]. The asymmetric hydroxylation at the benzylic position of 2,3-dihydrobenzopyran to produce the cyclic compound by microbial reaction was achieved in adequate yield and enantioselectivity by Boyd *et al.*[16] The asymmetric hydroxylation of the carbon chain of an alkylbenzene has been characterized. Holland *et al.*[17] have reported that, when *p*-diethylbenzene was employed as a substrate and *Helminthosporium* sp. as a microbe, the corresponding *R*-formed benzylalcohol was obtained in 97% ee and 10% yield. The enantioselectivity was not controlled completely and the yield was reduced as a result. The resolution of the racemic precursors of phthalides using lipase catalysts has not been studied [(3) in Scheme 1]. We attempted the organic synthesis of the precursors of the phthalides such as O-protected benzylalcohol-2-carboxylic acid, benzylalcohol-2-carboxylate, *o*-cyanobenzylalcohol, *o*-halobenzylalcohol, and *o*-amidobenzylalcohol. The synthetic method was problematic and when the compounds were synthesized, only racemic phthalides were obtained using the lipase catalyst.

We report that a complete asymmetric synthesis of the (*S*)-3-alkylphthalides, which are pharmacological active and found in *Angerica sinensis* Diels and *Sligusticum wallichii* Franch, was achieved for the first time by the microbial reduction caused by methyl 2-acylbenzoates and microbial hydroxylation caused by 2-alkylbenzoic acids.

Results and discussion

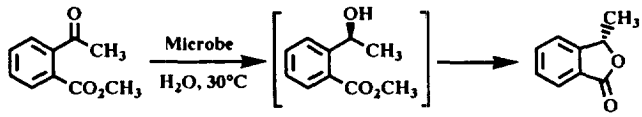
Asymmetric reduction of methyl 2-acylbenzoate, which is a key step for the production of alkylphthalide derivatives after cyclization was investigated. Methyl 2-acylbenzoates were prepared from phthalic anhydride with dialkylcadmium of one-, four-, or eight-carbon chains, followed by esterification in acidic methanol (Scheme 2).



Scheme 2.

The microbial reduction of methyl 2-acetylbenzoate selected as a basic structure was examined in water (Scheme 3). The culture medium was prepared from K_2HPO_4 (3.12 g), KH_2PO_4 (11.2g),

glycerol (30 g), polypepton (5.0 g), and yeast extract (10 g). The total volume was brought up to 1000 ml and the pH was adjusted to 6.8 and incubated at 30°C for 2 days. A solution of methyl 2-acetylbenzoate (0.1 g), 30 ml of distilled water, and 5.0 g of each microbe was stirred in the incubator at 30°C. The progress of the reaction was confirmed by gas chromatographic analysis.



Scheme 3.

Among the 30 strains of microbes examined, eight strains of the microbes were active in the asymmetric reduction of methyl 2-acetylbenzoate. Baker's yeast produced a 56% yield and 68% ee after incubation for 4 days at 30°C. In comparison with baker's yeast, the reactions were more selective when *Geotrichum* sp., *Mucor* sp. or *Endomyces* sp. was used (Table 1).

Although the enantioselectivity was over 99% ee for all microbial reactions except for baker's yeast, there were differences between the reaction times (Figure 1) and yields. The results suggested that the difference in the reaction times was due to the difference between the K_m or V_{max} of the enzymes which performed the synthesis in the corresponding microbes, and the longer the reaction time was, the more easily the degradation enzyme acted on the substrate. *Geotrichum candidum* (GC) IFO 34614 catalyzed this reaction in 1 day, which was the fastest time among the microbes examined, and produced (*S*)-3-methyl phthalide in 92% yield and 99% ee. This result was superior to the other methods in terms of reaction time, yield, and ee. The ee of 3-methylphthalide was determined to be over 99% by high performance liquid chromatographic analysis using a chiral column (DAICEL CHIRALCEL OB) because of the rapid cyclization after the asymmetric reduction.

The absolute configuration of 3-methylphthalide was determined by optical rotation, which was compared with the data of Ohkuma *et al.*[12] The reduction of methyl 2-pentanoylbenzoate and methyl 2-nonanoylbenzoate by GC 34614 was performed, but the yields were less than 1%. Methyl 2-acylbenzoates, which had long carbon chains, did not bind to the reaction site in the enzyme pocket.

The reaction of 2-acetylbenzoic acid with GC 34614 did not proceed at all. This suggested that the equilibrium of the 2-acylbenzoic acid, a keto acid, was optimal as the 3-hydroxy-3-alkylphthalide form in the solvent. It was confirmed that a peak by the hydroxyl proton in the carboxylic acid was not produced, but the single peak of the other hydroxyl proton in 3-hydroxy-3-alkylphthalide

Table 1. The synthesis of 3-methylphthalide by microbial reduction in H_2O[a]

Microbe	Time (days)	Temp. (°C)	Conv.(Yield) (%)	ee (%)	Config.
Geotrichum Candidum IFO4597	4	30	>99(70)	>99	*S* [c]
Geotrichum Candidum IFO5767	3	30	>99(55)	>99	*S*
Geotrichum Candidum IFO34614	1	30	>99(92)	>99	*S*
Mucor Javanicus IAM6087	10	30	>99(44)	>99	*S*
Mucor Javanicus IAM6101	5	30	>99(26)	>99	*S*
Mucor Heimalis IAM6095	10	30	>99(45)	>99	*S*
Endomyces magnusii IFO4600	10	30	>99(46)	>99	*S*
Endomyces resii IFO1112	2	30	>99(80)	>99	*S*
Bakers' Yeast [b]	4	30	>99(56)	68	*S*

a) Subst. 0.1g, Microbe 5g, H_2O 30ml, OV101 (Inj.180°C, Col. 130°C)
b) Subst. 0.1g, B.Y. 5g, H_2O 30ml, Glucose 1g
c) $[\alpha]_D$(23.0°C)= -46.4°(c=0.5, CH_2Cl_2)

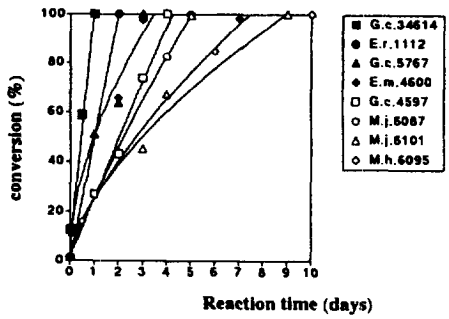


Figure 1. Time course for the microbial reduction of methyl 2-acetylbenzoate.

existed around 4.0 ppm by ^{1}H NMR analysis. Based on the present results, it is necessary to convert carboxylic acid to its corresponding ester to use it as a substrate. We studied the reactivity of methyl 2-acetylbenzoate in organic solvent using GC 34614. If the reaction proceeds in organic solvent, then it is practical to isolate the product, because the solvent can be removed and extraction is not necessary. Reduction of the substrate which is insoluble in water is possible.

In the organic solvent, the reduction did not proceed using an unmodified microbe. If the reduction proceeded, then the enantioselectivity should change.[18] Acetone, AcOEt, DMF, hexane, THF, toluene, propyl ether, isopropyl ether, butyl ether, or dioxane were employed as solvents to study the reactivity and the selectivity of the asymmetric reduction of methyl 2-acetylbenzoate, which proceeded only in butyl ether for 72 h to produce (*S*)-3-methylphthalide in 20% yield and 99% ee.

To increase the yield, we investigated the effect of phosphate buffer (pH 6.9) and the water content in butyl ether on the chemical yield and ee of the asymmetric reduction of methyl 2-acetylbenzoate using GC 34614. Phosphate buffer did not cause a significant change but the addition of H_2O did, as shown in Figure 2. The organic solvent that facilitated the asymmetric reduction of methyl 2-acylbenzoate was revealed. If the substrate is soluble only in organic solvents, then we recommend that the substrate should be dissolved in the minimum amount of butyl ether and added to water.

We next studied the asymmetric hydroxylation of alkylbenzoic acids using a microbe. Asymmetric hydroxylation is a useful organic reaction, because it can directly induce the functional oxygen group to a simple structure such as hydrocarbon. 2-Alkylbenzoic acids were selected as the hydroxylation substrates to obtain optically active 3-alkylphthalides.

In this study, three kinds of substrates, 2-ethylbenzoic acid, 2-pentylbenzoic acid, and 2-nonylbenzoic acid were prepared as shown in Scheme 4; an alkyliodide was added to a solution of *o*-toluic acid and *sec*-BuLi at −78°C to produce 83%, 95%, and 98% yields of the products, respectively.

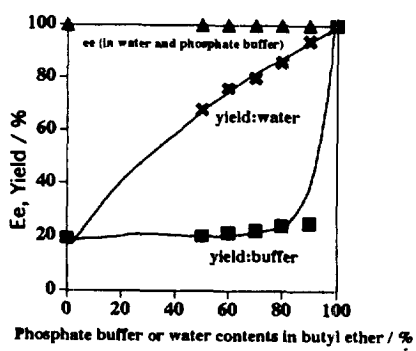
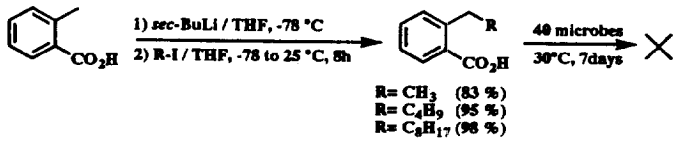
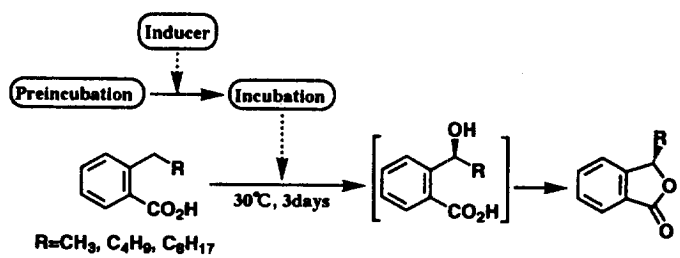


Figure 2. The effect of phosphate buffer or water content (v/v%) with butyl ether on the chemical yield and ee of the reduction of methyl 2-acetylbenzoate.



Scheme 4.

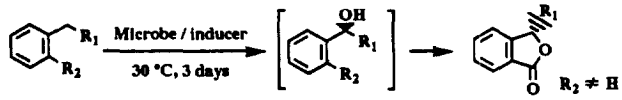
Microbial hydroxylation of 2-ethylbenzoic acid was examined in the same manner as microbial reduction. Forty kinds of microbes, of the *Aspergillus* sp., *Bacillus* sp., *Candida* sp., *Endomyces* sp., *Geotrichum* sp., *Mucor* sp., and *Pseudomonas* sp. groups, were examined for their ability to produce hydroxylation. The culture medium was prepared from K_2HPO_4 (3.12 g), KH_2PO_4 (11.2 g), glycerol (30 g), polypepton (5.0 g), and yeast extract (10 g) for *Aspergillus* sp., *Endomyces* sp. *Geotrichum* sp., and *Mucor* sp. The total volume was brought up to 1000 ml, the pH was adjusted to 6.8, and then incubated at 30°C for 2 days. The culture medium was prepared from $(NH_4)_3PO_4$ (15.0 g), KH_2PO_4 (7.0 g), glucose (40 g), $MgSO_4 \cdot 7H_2O$ (0.8 g), $ZnSO_4 \cdot 7H_2O$ (60 mg), $CuSO_4 \cdot 5H_2O$ (5 mg), $MnSO_4 \cdot 4H_2O$ (10 mg), and NaCl (0.1 g) for *Candida* sp. The total volume was brought up to 1000 ml, the pH was adjusted to 7.2, and then incubated at 30°C for 2 days. The culture medium was prepared from K_2HPO_4 (2.0 g), KH_2PO_4 (2.0 g), glycerol (2.0 g), polypepton (15.0 g), yeast extract (2.0 g), meat extract (2.0 g), and $MgSO_4 \cdot 7H_2O$ (0.1 g) for *Bacillus* sp. The total volume was brought up to 1000 ml, the pH was adjusted to 7.2 and then incubated at 55°C for 2 days. The culture medium was prepared from KH_2PO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), glucose (20 g), polypepton (5.0 g), and yeast extract (2.0 g) for *Pseudomonas* sp. The total volume was brought up to 1000 ml, the pH was adjusted to 7.2 and then incubated at 30°C for 2 days. A mixture of 2-ethylbenzoic acid (0.1 g), distilled water (30 ml) and 5.0 g of each microbe was stirred in the incubator at 30°C for 3 days. The progress of the reaction was monitored by gas chromatographic analysis. None of the reactions proceeded and the substrate was recovered. There was no reaction with the *Pseudomonas* sp microbe whose oxidative activity is known,[19a,b] therefore the additive effects of the inducers were examined. After preincubation, 40 mg/liter of a solution of *o*-toluic acid and 2-ethylbenzoic acid was added to the medium as an inducer (Scheme 5). The results showed that two microbes, *Pseudomonas putida* ATCC 12633 and *Aspergillus niger* IFO 6661, promoted hydroxylation. The two inducers were used separately to study the hydroxylation promoted by *Pseudomonas putida* ATCC 12633 and *Aspergillus niger* IFO 6661.



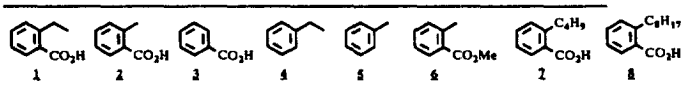
Scheme 5.

o-Toluic acid promoted the hydroxylation and the enantioselectivity was specific to obtain (*S*)-methylphthalide in 80% and 19% yield and 99% ee, respectively. It should be noted that the active enzyme was derived from the inducer which was *not the substrate*. The additive effects of other *o*-toluic acid derivatives, which were employed as inducers, were examined. When benzoic acid, ethylbenzene, or toluene was employed as an inducer, the hydroxylation did not proceed at all. The hydroxylation of ethylbenzene using *o*-toluic acid or ethylbenzene as the inducer, or that of methyl 2-ethylbenzoate using *o*-toluic acid or methyl 2-ethylbenzoate as the inducer did not occur. 2-Pentylbenzoic acid and

Table 2. Additive effects of inducers on microbial hydroxylation



Microbe	R_1	R_2	Inducer [a]	Yield.(%)	e.e.(%)
P. putida	CH_3	CO_2H	1	0	0
	CH_3	CO_2H	2	80	99[b]
	CH_3	CO_2H	3	0	0
	CH_3	CO_2H	4	0	0
	CH_3	CO_2H	5	0	0
	CH_3	CO_2Me	2	0	0
	CH_3	CO_2Me	6	0	0
	CH_3	H	4	0	0
	CH_3	H	2	0	0
	C_4H_9	CO_2H	2	12	99[b]
	C_4H_9	CO_2H	7	0	0
	C_8H_{17}	CO_2H	2	10	99[b]
	C_8H_{17}	CO_2H	8	0	0
A. niger	CH_3	CO_2H	1	0	0
	CH_3	CO_2H	2	19	99[b]

a) 

1 2 3 4 5 6 7 8

b) *S*-form

2-nonylbenzoic acid using *o*-toluic acid were hydroxylated at the benzylic position to produce (*S*)-butylphthalide and (*S*)-octylphthalide in 12% and 12% yield and 99% ee, respectively (Table 2).

The asymmetric hydroxylation of 2-alkylbenzoic acid proceeded using either *Pseudomonas putida* ATCC 12633 or *Aspergillus niger* IFO 6661, and it was induced by *o*-toluic acid. The enantioselectivity of the hydroxylation at the benzylic position was specific.

To our knowledge, *o*-toluic acid does not occur in a metabolic system. This result might be elucidated by the isolation of the inducing enzyme.

Conclusion

Optically active phthalide derivatives were synthesized in efficient yields and enantioselectivities by asymmetric reduction and hydroxylation using a microbial reaction. (*S*)-3-Alkylphthalide derivatives were synthesized by the asymmetric reduction of methyl 2-acetylbenzoate with *Geotrichum candidum* IFO 34614 in 99% ee and by the asymmetric hydroxylation of 2-alkylbenzoic acids at the benzylic position with either *Pseudomonas putida* ATCC 12633 or *Aspergillus niger* IFO 6661, whose fermentation was induced by *o*-toluic acid. These methods are applicable to the asymmetric reduction and hydroxylation promoted by other substrates.

Experimental

Instruments

^{1}H NMR spectra were recorded on a JEOL EX-270 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as the internal reference. IR spectra were recorded on a Hitachi 270-30 infrared spectrometer. Optical rotation was measured with a Horiba SEPA-200 polarimeter. Gas chromatographic analyses were performed using a Shimadzu gas chromatograph model GC-9A equipped with OV101 and a GL Science gas chromatograph model GC 353 equipped with TC-1. High-performance liquid chromatography analyses were performed using a Shimadzu liquid chromatograph model LC-10 equipped with DAICEL CHIRALCEL OB.

Materials

Organic reagents and lipases were purchased from commercial sources unless otherwise indicated.

Media and culture conditions

The following media were used. A medium was prepared from K_2HPO_4 (3.12 g), KH_2PO_4 (11.2 g), glycerol (30 g), polypepton (5.0 g), and yeast extract (10 g). The total volume was brought up to 1000 ml and the pH was adjusted to 6.8. After sterilization, 10 ml of this medium in a 100 ml test tube was inoculated with bacteria from stock cultures (*Geotrichum candidum* IFO 4597, *Geotrichum candidum* IFO 5767, *Geotrichum candidum* IFO 34614, *Endomyces magnusii* IFO 4600, *Endomyces resii* IFO 1112, *Mucor javanicus* IAM 6087, *Mucor javanicus* IAM 6101, *Mucor heimalis* IAM 6095). The test tubes were shaken for 2 days at 30°C, and then the medium was transferred into the 500 ml Sakaguchi flask and shaken for 1 day at 30°C.

2-Acetylbenzoic acid

Methyl bromide (7.6 g, 80 mmol) in 40 ml of dry THF was added dropwise to metallic Mg (2.2 g, 90 mmol) in 10 ml of dry THF. The mixture was stirred for 15 min at 45°C, then added to $CdCl_2$ (7.3 g, 40.0 mmol). The solution was stirred and refluxed for an additional 0.5 h, then 40 ml of 1 N HCl was added to the solution at 0°C. The mixture was poured into 50 ml of water and the organic material was extracted using ethyl acetate (3×80 ml). The combined organic layer was washed with 0.9% NaCl (3×80 ml), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was applied to column chromatography over silica gel with a 3:1 mixture of hexane and ethyl acetate as an eluent to afford 2-acetylbenzoic acid (4.5 g) in 69% yield. ^{1}H NMR ($CDCl_3$): δ; 1.92 (s, 3H), 3.30 (br, 1H), 7.56 (dd, 2H, J=9.57 and 7.59 Hz), 7.71 (dd, 1H, J=7.59 and 7.59 Hz), and 7.85 (d, 1H, J=7.59 Hz). IR (neat): 3383, 1750, and 1482 cm^{-1}. Calculated for the amount of $C_9H_8O_3$: C, 65.85; H, 4.91%. Found: C, 65.73; H, 4.88%.

2-Pentanoylbenzoic acid

This compound was obtained in the same manner as above in 64% yield. ^{1}H NMR ($CDCl_3$): δ; 0.87 (t, 3H, J=6.93 Hz), 1.23–1.36 (m, 4H), 2.04–2.21 (m, 2H), 3.32 (br, 1H), 7.58 (dd, 2H, J=9.57 and 7.59 Hz), 7.72 (dd, 1H, J=7.59 and 7.59 Hz), and 7.85 (d, 1H, J=7.59 Hz). IR (neat): 3500, 1762 and 1484 cm^{-1}. Calculated for the amount of $C_{12}H_{14}O_3$: C, 69.89; H, 6.84%. Found: C, 69.81; H, 6.83%.

2-Nonanoylbenzoic acid

This compound was obtained in the same manner as above in 57% yield, mp: 53.6–54.4°C. ^{1}H NMR ($CDCl_3$): δ; 0.87 (t, 3H, J=6.94 Hz), 1.21–1.37 (br, 12H), 2.02–2.29 (m, 2H), 3.36 (br, 1H), 7.58 (dd, 2H, J=9.57 and 7.59 Hz), 7.72 (dd, 1H, J=7.59 and 7.59 Hz), and 7.85 (d, 1H, J=7.59 Hz). IR (neat): 3500, 1762 and 1484 cm^{-1}. Calculated for the amount of $C_{16}H_{22}O_3$: C, 73.25; H, 8.45%. Found: C, 73.45; H, 8.39%.

Methyl 2-acetylbenzoate

A solution of 2-acetylbenzoic acid (6.26 g, 38.1 mmol) in 30 ml of MeOH was added dropwise to 0.1 ml of 97% H_2SO_4 at room temperature. The solution was stirred and refluxed for 1 h, then the organic solvent was removed and the organic material was extracted with ethyl acetate (3×80 ml). The combined organic layer was washed with saturated sodium hydrogencarbonate (3×80 ml), 0.9% NaCl (3×80 ml), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was applied to column chromatography over silica gel with a 4:1 mixture of hexane and ethyl acetate as an eluent to afford methyl 2-acetylbenzoate (3.6 g) in 53% yield. ^{1}H NMR ($CDCl_3$): δ; 2.55 (s, 3H), 3.90 (s, 3H), 7.42 (d, 1H, J=7.26 Hz), 7.50–7.58 (m, 2H), and 7.85 (d, 1H, J=7.58 Hz). IR (neat): 1724, 1703, 1435, 1358, and 1285 cm^{-1}. Calculated for the amount of $C_{10}H_{10}O_3$: C, 67.41; H, 5.66%. Found: C, 67.08; H, 5.54%.

Methyl 2-pentanoylbenzoate

This compound was obtained in the same manner as above in 55% yield. ^{1}H NMR ($CDCl_3$): δ; 0.88 (t, 3H, J=6.96 Hz), 1.21–1.33 (m, 4H), 2.42 (t, 3H, J=7.15 Hz), 3.90 (s, 3H), 7.41 (d, 1H, J=7.26 Hz), 7.52–7.62 (m, 2H), and 7.85 (d, 1H, J=7.58 Hz). IR (neat): 1725, 1703, 1436, 1358, and 1285 cm^{-1}. Calculated for the amount of $C_{13}H_{16}O_3$: C, 70.89; H, 7.32%. Found: C, 71.15; H, 7.33%.

Methyl 2-nonanoylbenzoate

This compound was obtained in the same manner as above in 50% yield. ^{1}H NMR ($CDCl_3$): δ; 0.89 (t, 3H, J=6.96 Hz), 1.20–1.37 (m, 12H), 2.42 (t, 3H, J=7.14 Hz), 3.92 (s, 3H), 7.41 (d, 1H, J=7.26 Hz), 7.50–7.62 (m, 2H), and 7.85 (d, 1H, J=7.58 Hz). IR (neat): 1724, 1703, 1435, 1358, and 1283 cm^{-1}. Calculated for the amount of $C_{17}H_{24}O_3$: C, 73.88; H, 8.75%. Found: C, 73.72; H, 8.82%.

Asymmetric synthesis of (S)-3-methylphthalide by microbial reduction

A mixture of methyl 2-acetylbenzoate (0.1 g), 30 ml of distilled water, and 5.0 g of *Geotrichum candidum* IFO34614 was stirred in an incubator at 30°C. A final check of the reaction was made by gas chromatographic analysis. The mixture was filtered and extracted with ethyl acetate (3×80 ml). The combined organic layer was washed with 0.9% NaCl (3×80 ml), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was applied to column chromatography over silica gel with a 4:1 mixture of hexane and ethyl acetate as an eluent to afford (*S*)-3-methylphthalide at a 92% yield. $[\alpha]_D^{23}$ −46.4 (c=0.50, CH_2Cl_2, ee >99%): lit.[12] $[\alpha]_D^{20}$ −31.6 (c=0.86, CH_2Cl_2, ee=99%) ^{1}H NMR ($CDCl_3$): δ; 1.64 (d, 3H, J=1.00 Hz), 5.60 (q, 1H, J=6.60 Hz), 7.44 (dd, 1H, J=1.0 and 7.59 Hz), 7.53 (dd, 1H, J=7.26 and 7.59 Hz), 7.68 (ddd, 1H, J=1.0, 7.26, and 7.59 Hz), and 7.90 (d, 1H, J=7.59 Hz). IR (neat): 2932, 1716, and 1526 cm^{-1}. Calculated for the amount of $C_9H_8O_2$: C, 72.96; H, 5.44%. Found: C, 72.79; H, 5.36%.

2-Ethylbenzoic acid

To a solution of *o*-toluic acid (10.0 g, 73.4 mmol) in dry THF (200 ml) at −78°C was added *sec*-BuLi (1.3 M solution in hexane, 123 ml, 0.16 mol) over a 2 h period. The solution was stirred at −78°C for 2 h, and then methyl iodide (52.5 g, 0.37 mol) was added. After being stirred at room temperature for 8 h, the reaction was quenched with a 10% solution of hydrochloric acid. Most of the organic solvents were removed at reduced pressure. The residue was diluted with water and then extracted twice with ethyl acetate (2×80 ml). The combined organic layers were washed with a 10% hypotonic solution and a saturated solution of sodium bicarbonate (3×80 ml) and brine (3×80 ml) and then dried over dry sodium sulfate. After concentration at reduced pressure, the residue was subjected to column chromatograpy over silica gel with a 3:1 mixture of hexane and ethyl acetate as an eluent to afford 2-methylbenzoic acid (9.1 g) in 83% yield. ^{1}H NMR ($CDCl_3$): δ; 1.26 (t, 3H, J=7.50 Hz), 3.00 (t, 2H, J=7.59 Hz), 7.20–7.40 (m, 2H), 7.48 (t, 2H, J=7.70 Hz), 8.00 (dd, 1H, J=1.30 and 1.80 Hz). IR (neat): 2956, 1692, and 1404 cm^{-1}. Calculated for the amount of $C_9H_{10}O_2$: C, 71.98; H, 6.71%. Found: C, 72.16 H, 6.61%.

2-Pentylbenzoic acid

This compound was obtained in the same manner as above in 95% yield. ^{1}H NMR ($CDCl_3$): δ; 0.89 (t, 3H, J=7.83 Hz), 1.20–1.40 (m, 4H), 1.50–1.74 (m, 2H), 7.20–7.30 (m, 2H), 7.41–7.53 (m, 1H), 8.00 (dd, 1H, J=0.7 and 1.60 Hz). IR (neat): 2956, 1693, and 1404 cm^{-1}. Calculated for the amount of $C_{12}H_{16}O_2$: C, 74.97; H, 8.39%. Found: C, 74.70; H, 8.57%.

2-Nonylbenzoic acid

This compound was obtained in the same manner as above in 98% yield. ^{1}H NMR ($CDCl_3$): δ; 0.82 (t, 3H, J=6.60 Hz), 1.20–1.40 (m, 12H), 1.50–1.78 (m, 2H), 2.98 (t, 2H, J=7.70 Hz), 7.31–7.44 (m, 2H), 7.42 (t, 1H, J=7.26 Hz), 8.00 (dd, 1H, J=1.0 and 1.6 Hz). IR (neat): 2950, 1700, and 1440 cm^{-1}. Calculated for the amount of $C_{16}H_{24}O_2$: C, 77.38; H, 9.74%. Found: C, 77.50; H, 9.69%.

Asymmetric synthesis of (S)-3-methylphthalide by microbial hydroxylation

Culture medium with *Pseudomonas putida* ATCC 12633 was prepared from KH_2PO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), glucose (20 g), polypepton (5.0 g), and yeast extract (2.0 g). The total volume was brought up to 1000 ml and the pH was adjusted to 7.2, and preincubated at 30°C for 1 day. After preincubation, 40 mg/liter of a solution with *o*-toluic acid as the inducer was added to the medium and it was incubated at 30°C for 2 days. A mixture of 2-ethylbenzoic acid (30 mg), 30 ml of distilled water, and 5.0 g of *Pseudomonas putida* ATCC 12633 was stirred in the incubator at 30°C. A final check of the reaction was made by gas chromatographic analysis. The mixture was filtered and extracted with ethyl acetate (3×80 ml). The combined organic layer was washed with 0.9% NaCl (3×80 ml), dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was applied to column chromatography over silica gel with a 4:1 mixture of hexane and ethyl acetate as an eluent to afford (*S*)-3-methylphthalide in 80% yield. $[\alpha]_D^{23}$ −46.4 (c=0.90, CH_2Cl_2, ee >99%): lit.[12] $[\alpha]_D^{20}$ −31.6 (c=0.86, CH_2Cl_2, ee=99%). The ^1H NMR data was obtained in the same manner as above.

(S)-3-Butylphthalide

$[\alpha]_D$ −71.3 (c=0.23, $CHCl_3$, ee >99%): lit.[11] $[\alpha]_D$ −49.3 (c=0.40, $CHCl_3$). ^1H NMR ($CDCl_3$): δ; 0.91 (t, 3H, J=7.36 Hz), 1.35–1.50 (m, 4H), 1.75–1.82 (m, 1H), 2.02–2.07 (m, 1H), 5.48 (dd, 1H, J=4.29 and 7.92 Hz), 7.43 (d, 1H, J=7.59 Hz), 7.53 (dd, 1H, J=7.26 and 7.26 Hz), 7.67 (dd, 1H, J=7.26, and 7.59 Hz), and 7.90 (d, 1H, J=7.59 Hz). IR (neat): 2932, 1716, and 1526 cm^{-1}. Calculated for the amount of $C_{12}H_{14}O_2$: C, 75.76; H, 7.42%. Found: C, 75.68; H, 7.39%.

(S)-3-Octylphthalide

$[\alpha]_D^{23}$ −53.1 (c=0.50, $CHCl_3$, ee >99%). ^1H NMR ($CDCl_3$): δ; 0.88 (t, 3H, J=6.93 Hz), 1.26–1.58 (br, 12H), 1.71–1.83 (m, 1H), 1.98–2.10 (m, 1H), 5.48 (dd, 1H, J=3.96 and 7.92 Hz), 7.43 (dd, 1H, J=1.01 and 7.59 Hz), 7.52 (dd, 1H, J=7.26 and 7.59 Hz), 7.67 (ddd, 1H, J=1.01, 7.26, and 7.59 Hz), and 7.90 (d, 1H, J=7.59 Hz). IR (neat): 2932, 1716, and 1526 cm^{-1}. Calculated for the amount of $C_{16}H_{22}O_2$: C, 78.01; H, 9.00%. Found: C, 77.89; H, 9.01%.

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