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Potential Antiinflammatory Agents. V.[1] Synthesis of Metabolites of 6-Chloro-5-cyclohexylindan-1-carboxylic Acid (TAI-284) using Microbiological Hydroxylation[2,3]

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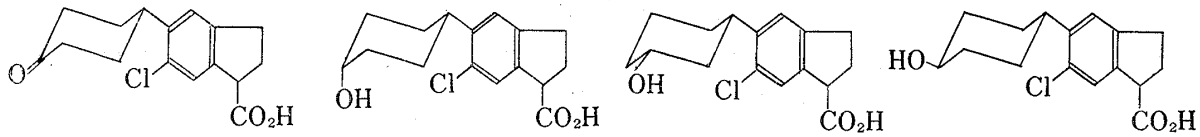
Microbiological hydroxylation of 6-chloro-5-cyclohexylindan-1-carboxylic acid (TAI-284), a new potent antiinflammatory agent, with *Penicillium concavo-rugulosum* IFO 6226 gave 6-chloro-5-(*trans*-4′-hydroxycyclohexyl)indan-1-carboxylic acid (**1**), one of the main metabolites of TAI-284 in rats, in 71% yield. From this product two other metabolites, the corresponding 4′-oxo and cis-4′-hydroxy derivatives (**4** and **2**), were derived chemically. Catalytic hydrogenation of the 4′-oxo compound (**4**) over PtO_2 afforded the unexpected *trans* alcohol (**1**), but reduction of **4** with trimethylphosphite and iridium tetrachloride in aqueous isopropanol gave the desired *cis* alcohol (**2**) in 69% yield.

Previously,[5] biotransformation of 6-chloro-5-cyclohexylindan-1-carboxylic acid (TAI-284), a new nonsteroidal antiinflammatory agent, was studied in isolated perfused rat liver and five metabolites, shown in Fig. 1, were isolated and identified.



4′-one **4** metabolite I

cis-4′-ol **2** metabolite IIa

cis-3′-ol metabolites IIb and IV diastereoisomers

trans-4′-ol **1** metabolite III

Fig. 1. Metabolites of TAI-284 in Rats

In the previous paper,[1] we reported the synthesis of these metabolites, but this chemical synthesis was difficult and required many reaction steps with a poor overall yield. Interestingly, studies on biological activities of the synthetic metabolites demonstrated that all of these metabolites possessed antiinflammatory activity, especially metabolite IIa having the same order of activity as TAI-284 and much lower ulcerogenic activity than the parent compound.[6] These facts prompted us to provide a more efficient, simple method to prepare these active metabolites for further biological evaluation. The present paper describes the microbiological hydroxylation of TAI-284, which gave 6-chloro-5-(*trans*-4′-hydroxycyclohexyl)indan-1-carboxylic acid (metabolite III) (**1**) as a principal product, and the chemical conversion of **1**

1) Part IV: S. Kishimoto, T. Aono, Y. Araki, I. Minamida, K. Tanaka, and S. Noguchi, *Chem. Pharm. Bull.* (Tokyo), **22**, 2231 (1974).
2) A part of this work was presented at the 93rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1973.
3) Taken in part from the Ph.D. thesis of S. Kishimoto, Kyoto University, March, 1975.
4) Location: *Juso-Honmachi, Yodogawa-ku, Osaka.*
5) S. Tanayama, E. Tsuchida, and Z. Suzuoki, *Xenobiotica*, **3**, 643 (1973); Y. Kanai, T. Kobayashi, and S. Tanayama, *ibid.*, **3**, 657 (1973).
6) S. Kuzuna, N. Matsumoto, and K. Kawai, *Japan. J. Pharmacol.*, **24**, 687 (1974).

into the isomeric *cis*-4′-hydroxy compound (metabolite IIa) (**2**) and related 4′-substituted compounds.

Microbiological Hydroxylation of TAI-284

The most important process in the metabolism of TAI-284 is obviously hydroxylation on the cyclohexane ring. In the field of steroid chemistry, hydroxylation by microorganisms has proved to be an efficient preparative method in a number of cases.[7] This approach suggested that the desired hydroxylated metabolites might be produced readily by use of microbial enzymes. Initial screening of microorganisms capable of hydroxylating TAI-284 was carried out by using those supplied mainly from Institute for Fermentation, Osaka, and also chosen from our collection. About 3000 organisms were examined, including fungi (1600 strains), bacteria (800 strains) and actinomycetes (500 strains). Among them only fungi (about 400 strains) showed the ability to metabolize TAI-284. After further examination the formation of the *trans*-4′-hydroxylated compound (**1**) as a principal product was proved by thin-layer and gas chromatography (TLC and GLC) in the incubation mixtures of 40 strains listed in Table I. As shown in this table, TAI-284-hydroxylating enzymes were found to be distributed in the wide range of genera of fungi. Interestingly, **1** had also been found in animals as the major metabolite before.[5]

Table I. Strains of Fungi Selected for Conversion of TAI-284 into Its *trans*-4′-Hydroxy Derivative (**1**)

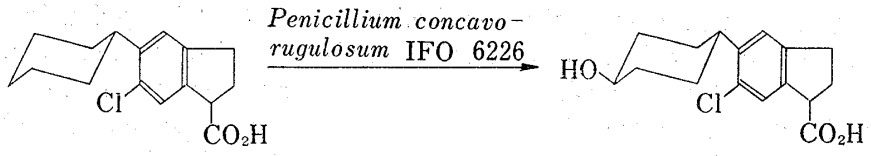
Absidia coerulea. IFO 4012	*Penicillium canescens* IFO 7594
absidia glauca. IFO 4003	*Penicillium charlesii* IFO 7524
Aspergillus luchuensis IFO 4281	*Penicillium concavo-rugulosum* IFO 6226
Aspergillus terreus var. africanus IFO 8835	*Penicillium corylophiloides* P 641
Botryosporium pulchrum IFO 6824	*Penicillium crustosum* P 557
Botryotinia fuckeliana IFO 7186	*Penicillium cyclopium* P 465
Cercospora abelmoschi IFO 6423	*Penicillium duclauxi* NRRL 2020[b]
Cladosporium coralloides IFO 6536	*Penicillium ochrosalmoneum* P 383
Epicoccum nigrum IFO 7808	*Penicillium oxalicum* IFO 7085
Eurotium repens IFO 4884	*Penicillium purpurrescens* IFO 6033
Fusarium roseum ATCC 10914	*Penicillium rugulosum* IFO 5746
Fusarium solani ATCC 12823	*Penicillium sp.* S-59-4
Helminthosporium dematioideum IFO 7364	*Penicillium sp.* 95—180
Lasiodiplodia theobromae SS 201	*Penicillium spinulosum* IFO 5723
Mucor griseo-cyanus IFO 4563	*Penicillium thomii* IFO 7988[a,b]
Mucor javanicus IFO 4570	*Penicillium trzebinskii* NRRL 731
Neurospora sitophila IFO 6070	*Porodisculus pendulus* IFO 4967
Oospora destructor IFO 8556	*Pyrenophora graminea* IFO 6633
Penicillium adametzi IFO 7680	*Stemphylium sp.* MD 427
Penicillium aurantio-violaceum NRRL 760[b]	*Verticillium niveostratosum* IFO 5435[b]

a) *cis*-4′-Hydroxy derivative (**2**) was also detected by TLC.
b) *cis*-3′-Hydroxy derivatives were also detected by TLC.

Of these strains in Table I *Penicillium concavo-rugulosum* IFO 6226 was chosen for the preparation of **1** on the basis of the result of TLC of the reaction mixture, which showed the almost complete conversion of TAI-284 and no formation of by-products. After the cultivation of this fungus for 2 days, the sodium salt of TAI-284 was added to the culture broth so as to make the final concentration 0.1% and the flask was shaken on a reciprocal shaker at 24°. After 7 days most of the substrate was consumed, and **1** was obtained from the etherial extracts as colorless crystals, mp 188—190°, in 71% yield. The structure was confirmed by comparison of infrared (IR), ultraviolet (UV), mass and nuclear magnetic resonance (NMR)

7) W. Charney and H.L. Herzog, "Microbial Transformations of Steroids. A Handbook," Academic Press, New York, N.Y., 1967.

spectra with those of an authentic sample[1] and mixed melting point determination. When the incubation was scaled up and carried out in a fermentor of 50 liters under aeration, the transformation finished within only 42 hr.



TAI-284 **1** metabolite III

Chart 1

In the experiments described above, (±)-TAI-284 was used as the substrate and the resulting product was also racemic. On the other hand, when (*R*)-(−)-TAI-284[8] was incubated with the same strain, (−)-6-chloro-5-(*trans*-4′-hydroxycyclohexyl)indan-1-carboxylic acid (**3**) was obtained as colorless crystals, mp 211—213°, $[\alpha]_D^{23}$ −25.0° (*c*=1, MeOH). The absolute configuration of **3** was assigned the rectus at C-1 by comparison of its optical rotatory dispersion spectrum with that of (*R*)-(−)-TAI-284 (Fig. 2). Thus it was considered that this fungus converted both (*R*)- and (*S*)-TAI-284 into the corresponding (*R*)- and (*S*)-*trans*-4′-hydroxy derivatives, respectively, without epimerization at C-1.

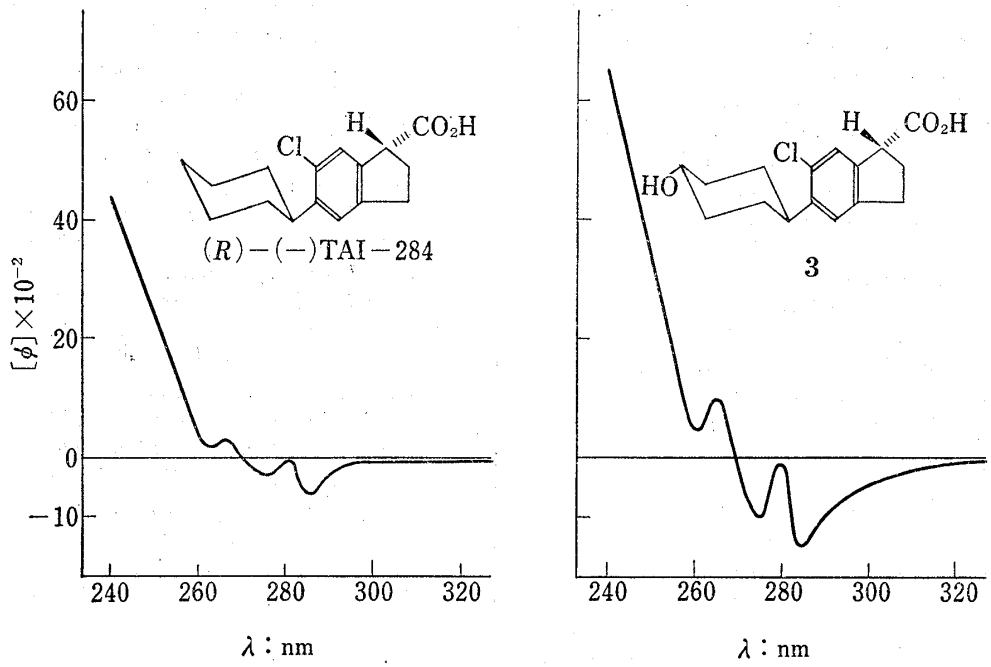


Fig. 2. Optical Rotatory Dispersion Curves of (*R*)-(−)-TAI-284 and (−)-*trans*-4′-Hydroxylated TAI-284 (**3**)

Other hydroxylated metabolites such as *cis*-4′- or *cis*-3′-hydroxy compounds were also detected by TLC in the incubation mixtures of TAI-284 with some fungi, but their amount was very small.

Chemical Transformation of 6-Chloro-5-(*trans*-4′-hydroxycyclohexyl)indan-1-carboxylic Acid (1) into Its *cis*-Isomer (2) and Related Compounds

Although **1** was obtained in a good yield by microbiological hydroxylation of TAI-284 as described above, the isomeric *cis*-4′-hydroxy compound (**2**) was not obtained efficiently by

8) S. Noguchi, S. Kishimoto, I. Minamida, and M. Obayashi, *Chem. Pharm. Bull.* (Tokyo), **22**, 529 (1974).

this direct hydroxylation. It was therefore attempted to convert **1** into **2** by chemical reactions. Oxidation of **1** with Jones reagent[9] gave 6-chloro-5-(4′-oxocyclohexyl)indan-1-carboxylic acid (**4**), mp 159.5°, which was identified with metabolite I[5] by means of IR, NMR and mass spectroscopy. This 4′-oxo compound (**4**) was subsequently hydrogenated over plutinum oxide in an acidic medium in expectation of the formation of the corresponding *cis*-axial alcohol (**2**). The predominant product, however, was the *trans*-equatorial alcohol (**1**) as shown in Table II. This result shows a sharp contrast to that in the hydrogenation of 5-(4′-oxocyclohexyl)indan-1-carboxylic acid (**5**), the dechloro analogue of **4**, which gave the corresponding *cis*-axial alcohol (**6**) by the similar reduction.[1] Sodium borohydride reduction or catalytic hydrogenation in a neutral medium also resulted in the more predominant formation of **1** (Table II).

Table II. Catalytic and Sodium Borohydride Reduction of 6-Chloro-5-(4′-oxocyclohexylindan)-1-carboxylic Acid (4)

Reduction conditions	Product composition[a] (*cis*-axial alcohol %)
H_2, PtO_2 in HCl–AcOH	29
H_2, PtO_2 in AcOH	20
H_2, PtO_2 in AcOEt	9
$NaBH_4$ in NaOH–H_2O	7

a) analyzed by gas chromatography

In general, catalytic hydrogenation of unhindered cyclohexanones in acidic medium proceeds from the less-hindered equatorial side so as to give an axial alcohol.[10] However, the carbonyl of **4**, which probably has a restricted conformation shown in Fig. 3 due to the interaction between the chlorine substituent at C-6 and axial hydrogens at C-2′ and C-6′,[1] seems to be somewhat hindered in the equatorial side by the chlorine. Thus it is primarily assumed that in the case of **4** the catalyst may be forced to approach to the carbonyl from the axial side leading to the predominant formation of the equatorial alcohol (**1**).

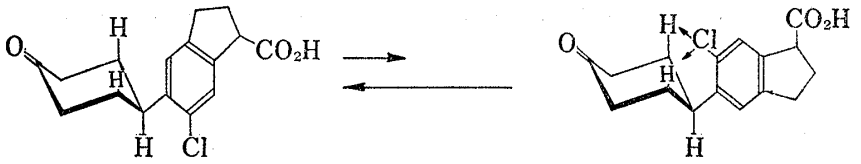


Fig. 3. Conformational Equilibration of 6-Chloro-5-(4′-oxocyclohexyl)indan-1-carboxylic Acid (**4**)

This assumption, however, seems to be inadequate for the interpretation of the result of the hydrogenation of **4**, because 6-chloro-5-(4′-methylenecyclohexyl)indan-1-carboxylic acid (**7**), which was prepared from **4** by the Wittig reaction and probably has a similar conformation as **4**, gave the corresponding axial methyl compound, 6-chloro-5-(*cis*-4′-methylcyclohexyl)-indan-1-carboxylic acid (**8**), by the similar catalytic hydrogenation: this result indicates that the chlorine substituent at C-6 has no influence on the direction of approach of catalyst to the substrate. The stereochemistry of the methyl group at C-4′ of **8** was confirmed by comparison of the NMR spectral data of **8** with those of *cis-trans* stereoisomeric 4-methyl-1-phenylcyclohexanes (**9** and **10**) reported by Garbish,[11] as shown in Fig. 4. Garbish reported that the axial methyl proton absorption in **9** appeared as a largely splitting doublet (J=6.9 Hz) at

9) K. Bowden, I.M. Heilbron, E.R.H. Jones, and B.C.L. Weedon, *J. Chem. Soc.*, **1946**, 39.
10) K. von Auwers, *Liebigs Ann.*, **420**, 91 (1920); A. Skita, *ibid.*, **431**, 1 (1923); D.H.R. Barton, *J. Chem. Soc.*, **1953**, 1027.
11) E.W. Garbisch, Jr. and D.B. Patterson, *J. Am. Chem. Soc.*, **85**, 3228 (1963).

lower field (1.01 ppm) than the equatorial one in **10** (0.92 ppm). The methyl absorption in **8** was observed at 1.05 ppm as a doublet (J=7.0 Hz), indicating that the methyl group at C-4′ is in the axial position. Although the effect of the chlorine substituent on the hydrogenation of **4** is still not clear, the chlorine may play a role at the late stage of the hydrogenation, probably in the half-hydrogenated state reported by Horiuti and Polanyi,[12] leading to the predominant formation of the more stable equatorial alcohol.

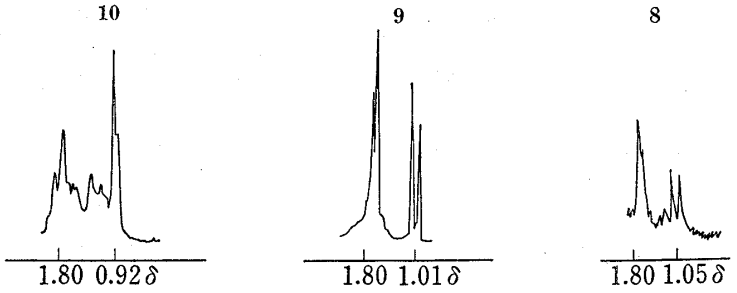
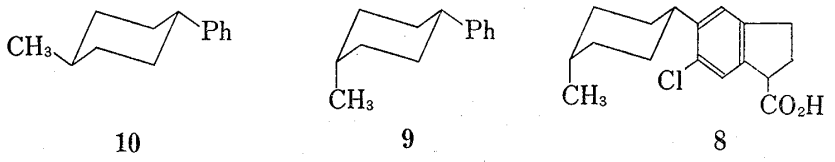


Fig. 4. High Field Parts of NMR Spectra of *cis*-and *trans*-4-Methyl-1-phenylcyclohexanes[10] (**9** and **10**) and 6-Chloro-5-(*cis*-4′-methylcyclohexyl)indan-1-carboxylic Acid (**8**)



10 9 8

The desired axial hydroxy compound (**2**) was finally obtained in 69% yield, mp 206—210°, after treatment of **4** with iridium tetrachloride and trimethylphosphite in aqueous isopropanol by the reduction method of Henbest, *et al.*, who have reported the stereoselective reduction of 4-*t*-butylcyclohexanone to the corresponding axial alcohol using the iridium-containing catalyst[13] (Chart 2). Details of the pharmacological study of **2** and related compounds prepared in the present paper will be published elsewhere by Kuzuna, *et al.*, biologists of our research division.

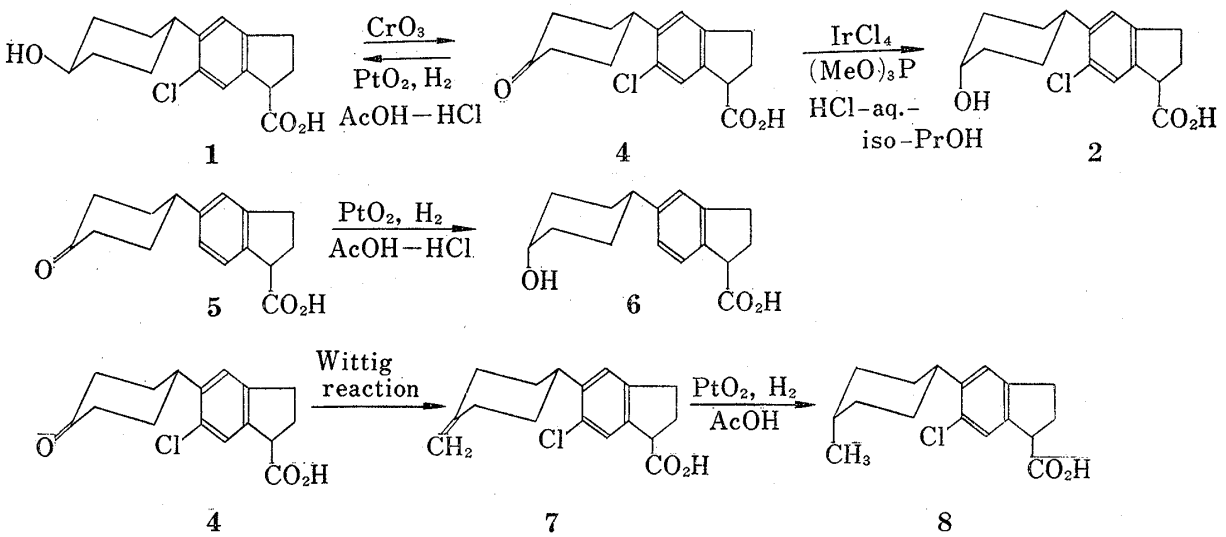


Chart 2

12) J. Horiuti and M. Polanyi, *Trans. Faraday Soc.*, **30**, 1164 (1934).
13) H.B. Henbest and T.R.B. Mitchell, *J. Chem. Soc.* (*C*), **1970**, 785.

Experimental[14]

Isolation of 6-Chloro-5-(*trans*-4′-hydroxycyclohexyl)indan-1-carboxylic Acid (1) Formed by Enzymatic Conversion of 6-Chloro-5-cyclohexylindan-1-carboxylic Acid (TAI-284) with *Penicillium concavo-regulosum* IFO 6226——a) Flask Experiment: A medium containing glucose (4%), sodium glutamate (0.5%), yeast extract (0.1%) and TAI-284 (0.001%) was prepared and adjusted to pH 5.0 with an aqueous solution of NaOH. Mycelia of *P. concavo-rugulosum* IFO 6226 grown on an agar slant were inoculated in four 300 ml flasks (each containing 50 ml of the sterilized medium) and the organism was grown at 24° for 2 days on a reciprocating shaker. The culture fluids were transferred into four 2-liter Sakaguchi flasks (each containing 500 ml of the sterilized medium) and the flasks were shaken at 24° for further 2 days. Then a solution of 500 mg of sodium salt of TAI-284 in 50 ml of water was added in each flask. After being shaken for additional 7 days the combined culture fluid was adjusted to pH 13 with 1N aqueous solution of NaOH, and was centrifuged. The resulting supernatant solution was adjusted to pH 2 with 1N HCl and extracted with ether. The extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in ether again and the solution was allowed to stand overnight at room temperature. The precipitate was collected, washed with ether and dried to give **1** as colorless crystals, mp 188—190° (1.42 g, 71.2%). This incubation product (**1**) was identified with an authentic sample[1] by comparison of IR, UV, NMR, and mass spectra and mixed melting point determination. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1710 (C=O), 1080 (C–OH). *Anal.* Calcd. for $C_{16}H_{19}O_3Cl$: C, 65.19; H, 6.50; Cl, 12.03. Found: C, 65.42; H, 6.46; Cl, 12.34. Recrystallization from acetone gave dimorphic colorless crystals, mp 199.5°. IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1700 (C=O), 1060 (C–OH). *Anal.* Calcd. for $C_{16}H_{19}O_3Cl$: C, 65.19; H, 6.50; Cl, 12.03. Found: C, 65.20; H, 6.54; Cl, 12.11. The NMR spectra of both the dimorphic crystals were identical.

b) Tank Experiment: A medium containing glucose (4%), sodium glutamate (0.5%), yeast extract (0.1%), peptone (0.4%) and TAI-284 (0.01%) was prepared and adjusted to pH 5.0 with an aqueous solution of NaOH. Mycelia of *P. concavo-rugulosum* IFO 6226 were inoculated in six 300-ml flasks (each containing 50 ml of the sterilized medium) and the organism was grown at 24° for 2 days on a reciprocating shaker. The culture fluids were transferred into six 2-liter Sakaguchi flasks (each containing 500 ml of the sterilized medium). After 2 days of further cultivation the contents of the flasks were transferred together into a 50-liter tank, which contained 30 liters of the sterilized medium. The organism was grown at 24° for 2 days with agitation and aeration, and then a sterilized solution of 60 g of sodium salt of TAI-284 in 3 liters of water was added to the mixture. After additional reaction for 2 days the culture fluid was adjusted to pH 12.5 with 20% aqueous solution of NaOH and mycelia were removed by filtration. The filtrate was adjusted to pH 2 with 12N HCl and then extracted twice with 15-liter portions of AcOEt. The combined extracts were concentrated to 2.5 liters under reduced pressure. The concentrate was extracted with 2% aqueous solution of NaOH, and the extract was washed with ether and then adjusted to pH 3 with 12N HCl. The resulting precipitate was extracted into ether and the etherial solution was treated as described above in the case a), giving **1** in the similar yield.

(*R*)-(—)-6-Chloro-5-(*trans*-4′-hydroxycyclohexyl)indan-1-carboxylic Acid (**3**)——A medium containing glucose (4%), sodium glutamate (0.5%), yeast extract (0.1%), peptone (0.4%) and (*R*)-(—)-TAI-284 (0.01%) was prepared and adjusted to pH 5.0 with an aqueous solution of NaOH. Mycelia of *P. concavo-rugulosum* IFO 6226 were inoculated in two 300 ml flasks (each containing 50 ml of the sterilized medium) and the organism was grown at 24° for 2 days on a reciprocating shaker. The culture fluids were transferred into two 2-liter Sakaguchi flasks (each containing 500 ml of the sterilized medium) and the flasks were shaken at 24° for further 2 days. Then a solution of 500 mg of sodium salt of (*R*)-(—)-TAI-284 in 50 ml of water was added in each flask. After being shaken for additional 6 days the combined culture fluid was made slightly alkaline with $NaHCO_3$ and mycelia were removed by filtration. The filtrate was adjusted to pH 2 with 1N HCl, and extracted with ether. The extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The solid residue was recrystallized 4 times from acetone to give **3** as colorless crystals, mp 211—213° (400 mg, 40%). *Anal.* Calcd. for $C_{16}H_{19}O_3Cl$: C, 65.19; H, 6.50; Cl, 12.03. Found: C, 65.31; H, 6.48; Cl, 12.20. IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH), 1720 (C=O). $[\alpha]_D^{25}$: −25.0° (c=1, MeOH).

6-Chloro-5-(4′-oxocyclohexyl)indan-1-carboxylic Acid (**4**)——To a stirred, ice-cooled solution of 1.0 g of **1** in 100 ml of acetone was added dropwise 2 ml of Jones reagent[9] in the period of 10 min. Stirring was continued for 30 min at 10—20°, and then 80 ml of water was added to the reaction mixture. Acetone was evaporated under reduced pressure and the resulting precipitate was collected and dissolved in AcOEt. The solution was washed successively with water, dilute HCl and water, dried over $MgSO_4$, and concentrated under reduced pressure. The solid residue was recrystallized from AcOEt to give **4** as colorless crystals, mp 159.5° (730 mg, 74%). *Anal.* Calcd. for $C_{16}H_{17}O_3Cl$: C, 65.64; H, 5.85; Cl, 12.11. Found: C, 65.50; H, 5.74; Cl, 12.32. IR ν_{max}^{KBr} cm^{-1}: 1710, 1680 (C=O).

14) Melting points were uncorrected. IR spectra were obtained with a Hitachi-215 spectrophotometer and NMR spectra with a Varian A-60 spectrometer using tetramethyl silane (TMS) as internal standard.

6-Chloro-5-(4′-methylenecyclohexyl)indan-1-carboxylic Acid (7)——After being washed twice with pentane, 0.36 g of the 50% oil suspension of NaH was added to 7 ml of dimethyl sulfoxide which had freshly been distilled over CaH_2. The suspension was stirred at 60—70° for 45 min and then cooled with ice–water. To the reaction mixture was added dropwise a solution of 2.6 g of methyl triphenylphosphonium bromide in 12 ml of dimethyl sulfoxide in the period of 3 min. The mixture was stirred for 10 min at room temperature. A solution of the methyl ester of **4**, which was prepared from **4** with diazomethane, in 10 ml of anhydrous tetrahydrofuran was then added dropwise to the mixture in the period of 15 min. After being stirred at room temperature for 3 hr, the reaction mixture was poured into water and extracted with ether. The extract was washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on silica gel with benzene–AcOEt (40: 1) to give the methyl ester of **7** as an oil. The ester was hydrolyzed in an alkaline solution and the resulting free acid was recrystallized from benzene–hexane to give **7** as colorless crystals, mp 138—140° (850 mg, 57%). *Anal.* Calcd. for $C_{17}H_{19}O_2Cl$: C, 70.22; H, 6.43; Cl, 12.19. Found: C, 70.13; H, 6.46; Cl, 12.08. IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O).

6-Chloro-5-(*cis*-4′-methylcyclohexyl)indan-1-carboxylic Acid (8)——One hundred mg of **7** was hydrogenated over 50 mg of PtO_2 in 10 ml of AcOH at room temperature. After 1 hr the catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The residual solid was recrystallized from hexane to give **8** as colorless crystals, mp 135—137° (60 mg, 60%). The structure of **8** was confirmed by the following spectral data: IR ν_{max}^{KBr} cm^{-1}: 1705 (C=O). NMR ($CDCl_3$) δ: 7.4 (1H, s, C_4–H), 7.2 (1H, s, C_7–H), 4.0 (1H, t, $J=7$ Hz, C_1-H), 2.9 (2H, m, C_3-H), 2.4 (2H, m, C_2-H), 1.1 (3H, d, $J=7$ Hz, CH_3).

6-Chloro-5-(*cis*-4′-hydroxycyclohexyl)indan-1-carboxylic Acid (2)——To 14.4 ml of water were added 0.4 ml of concentrated HCl, 0.32 g of $IrCl_4$ and 4.0 ml of trimethyl phosphite. The resulting solution was added dropwise to a solution of 3.0 g of **4** in 50 ml of iso–PrOH. The mixture was heated under reflux for 25 hr and concentrated under reduced pressure. Water was added to the residue and the precipitate was extracted with ether. The extract was washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in ether again and treated with an etherial solution of diazomethane. The resulting methyl ester was chromatographed on silica gel with benzene–AcOEt (4: 1) to give the methyl ester of **2** as an oil (2.4 g). The ester was hydrolyzed in an alkaline solution and the resulting free acid was recrystallized from acetone–benzene to give **2** as colorless crystals, mp 206—208° (2.1 g, 69%). *Anal.* Calcd. for $C_{16}H_{19}O_3Cl$: C, 65.19; H, 6.50. Found: C, 65.44; H, 6.25. The IR and NMR spectra of this compound were identical with those of an authentic sample.[1]

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