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THE ABSOLUTE STEREOCHEMISTRY OF SEVERAL *CIS*-DIHYDRODIOLS MICROBIALLY PRODUCED FROM SUBSTITUTED BENZENES

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Abstract—The absolute stereochemistry of a series of 3-substituted *cis*-dihydrodiols obtained by microbial oxidation of toluene, ethylbenzene, chlorobenzene, and biphenyl has been assigned from chemical and spectrophotometric studies. *p*-Halogenated toluenes also have been investigated as substrates in these oxidations, and the corresponding *cis*-dihydrodiols isolated. Implications from the observation that some of the *cis*-dihydrodiols are racemic are discussed. Oxidation of an olefin, (±)-3-methylcyclohexene, by these micro-organisms leads only to *cis*-diols whose absolute stereochemistry about the hydroxyl-bearing carbons is the same as that found for the *cis*-dihydrodiols formed from the aromatic substrates.

In a series of studies on the bacterial oxidation of aromatic hydrocarbons, Gibson *et al.*[2] have established that these compounds are oxidized to *cis*-dihydrodiols. These oxidations contrast with those of mammalian metabolic routes, which involve epoxides as intermediates and yield *trans*-dihydrodiols.[3] The enzyme system which forms the dihydrodiols discussed in the text incorporates atoms of an oxygen molecule into the cyclic substrates. Similar reactions have been observed in the bacterial oxidation of benzoic acid,[4a] styrene,[4b] and pyrazon.[4c] Since many of the *cis*-dihydrodiols obtained by microbial oxidation are optically active, it is reasonable to assume that the substrates are bound to the oxygenases in a stereospecific manner. In order to learn more concerning the factors which affect and/or determine this binding, we established the absolute stereochemistry of the dihydrodiols formed by oxidation of a series of substituted benzenes. No single approach for determining the absolute stereochemistry of these metabolites is available; consequently, a number of chemical and spectrophotometric methods were employed.

The *cis*-dihydrodiol, **1**, obtained from toluene[5] on oxidation by *Pseudomonas putida* was used as a model for both chemical transformations and spectrophotometric measurements. This metabolite was related to (−)-2(R)-methyladipic acid[6] by the chemical transformations outlined in Scheme 1.[7] Hydrogenation of **1** over Pd/charcoal in ethyl acetate containing triethylamine yielded a diastereomeric mixture of the *cis,cis* and *cis,trans* isomers. The predominant isomer formed by hydrogenation was purified via its monobenzoate **2b**, obtained through reaction of the crude hydrogenation mixture with one equivalent of benzoyl chloride in pyridine. The relative stereochemistry of **2b** was established as *cis,cis* from an analysis of its NMR spectrum,[7,8] which showed $J_{1,2} = J_{2,3} = 2.8$ Hz. Hydrolysis of **2b** gave the pure (−)-diol, which on oxidation with Jones reagent yielded (−)-2(*R*)-methyladipic acid, **4**. Since the relative stereochemistry of **2a** was known, and the absolute stereochemistry of the carbon-bearing Me group in **4** is (*R*), it follows that **2a** is (1*S*,2*R*)-dihydroxy-3(*R*)-methylcyclohexane and **1** is (1*S*,2*R*)-dihydroxy-3-methylcyclohexa-3,5-diene. An independent X-ray analysis[9] of the Diels–Alder adduct of the diacetate of **1** and *p*-bromophenyltriazolinedione confirmed the absolute stereochemistry established by the chemical method.

In order to determine the effect of changes in the size and nature of the benzene ring substituent on the absolute stereochemistry of the *cis*-dihydrodiols, we first examined the *cis*-dihydrodiol, **5**, obtained on oxidation of ethylbenzene[10] by *Pseudomonas putida*. The chemical approach successful for **1** could not be employed in this case, since 2-ethyladipic acid has not been resolved.

We have therefore employed spectrophotometric methods to relate **1** and **5**, as well as their *cis,cis*-tetrahydro derivatives. The CD data obtained for **1** and **5** are given in Table 1. Two approaches relating the sign of the longest wavelength transition of a diene to its absolute stereochemistry have been reported.[11] In the first, the optical activity of the molecule in question is considered to be dominated by a dissymmetric chromophore, the non-planar diene. Little attention is given to the nature of the quasi-axial and quasi-equatorial substituents on allylic carbons. More recent semi-empirical calculations[11c] have shown that this model as first proposed was too simple and that allylic substituents can make as important a contribution to the optical activity

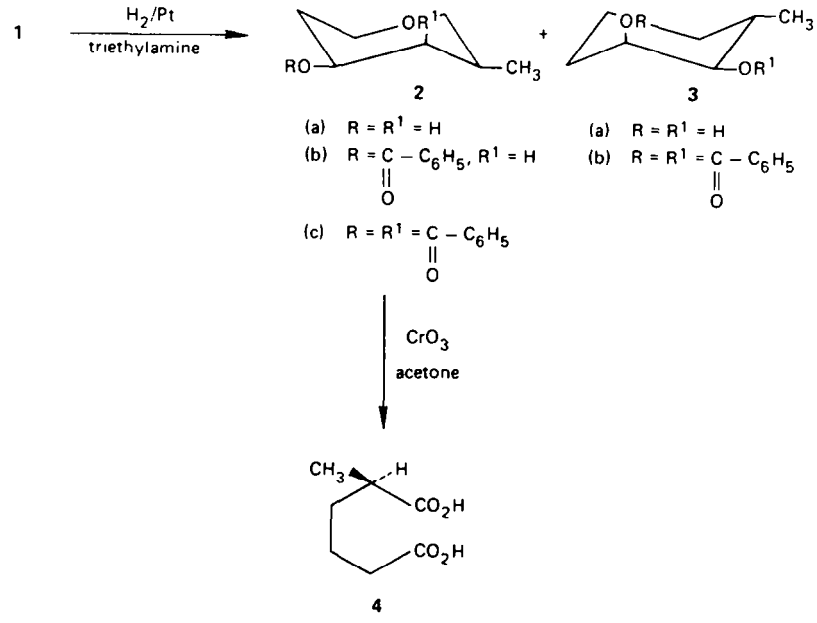
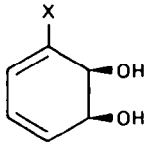


Table 1. Summary of CD measurements of dihydrodiols†

Starting Compound	Dihydrodiol Structure	θ	At wavelength nm	Absorption nm
Toluene	1	+ 1,985	270	266
Ethylbenzene	5	+ 2,680	277	266
Chlorobenzene	6	+ 3,590	280	271
p-Fluorotoluene	7	+17,750	265	266

†The CD measurements were made in ethanol on a Cary 60 spectropolarimeter.



1 X = CH_3
5 X = C_2H_5
6 X = Cl
11 X = C_6H_5

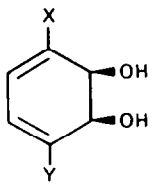
as that of the diene. In a second approach, several empirical descriptions relating the sign associated with the longest wavelength transition of the diene were proposed, each focusing on the nature and geometry of the allylic substituents. If one compares the CD bands for **1** and **5** and analyses the observed CD data (Table 1) by either approach it is necessary to assume that the preferred conformer or its relative rotatory strength has not changed in the two compounds. The analysis employing the first approach argues that the similarities in the sign and magnitude of the bands of these compounds required the dienes to have the same skew sense and therefore the compounds to have the same absolute stereochemistry. On the other hand, focusing on the allylic substituents (as the second approach does) since both compounds have two allylic OH groups and the same sign for the CD bands they have the same absolute stereochemistry. However, the necessity of assuming preferred conformations prompted us to deduce the absolute stereochemistry of the *cis,cis*-tetrahydro derivative of **5** from the sign of the CD curve of the dibenzoate in conjunction with the "Dibenzoate Chirality Rule" of Nakanishi and Harada.[13] Since the conformation of the dibenzoate is important in determining the CD pattern, it was essential to compare dibenzoates of the same geometric isomers of **1** and **5**. On hydrogenation of **1** and **5**, the *cis,cis* 3-substituted cyclohexane diol of each was purified and its stereochemistry assigned from an analysis of its proton NMR spectrum. The dibenzoates of the two *cis*-diols were prepared and the CD curves of both dibenzoates were found to exhibit negative bands at 235 nm and positive bands at 220 nm. In the case of monocyclic systems, energy differences between conformations can be small and the rotational strengths associated with different conformations can vary by an order of magnitude. However, the similarity in the shape and magnitude of the CD curves of the two dibenzoates suggests that they have the same absolute stereochemistry. Since both methods relating the sign of the CD curve with absolute stereochemistry use very different rules, the assignment seems quite secure.

In order to determine the effect of a halogen atom on the course of the oxidation, the *cis*-dihydrodiol produced from chlorobenzene by *P. putida* was examined. The sign and magnitude of the observed CD band (Table 1)

are similar to those of **1** and **5**. The conclusion that the absolute stereochemistry at the OH-bearing carbons of **6** is identical with that of **1** and **5** could not be verified, but the data strongly suggest that this is the case.

The amplitudes of the CD band of **1**, **5** and **6** are approximately one-fifth to one-quarter of those observed for other dienes,[11] suggesting the presence of a conformational equilibrium between the two possible conformers, as in α-phellandrene.[14] Both Snatzke *et al.*[15] and Horsman and Emeis[16] investigated the low-temperature CD curves of α-phellandrene and showed that the magnitude of the band decreased with decreasing temperature, finally changing sign below −150°. In order to determine whether an analogous situation existed for these *cis*-dihydrodiols, the low-temperature CD of **5** was measured. The curve is shown in Fig. 1, along with the curve at 25°; it differs considerably from that of α-phellandrene and the low-temperature CD curve of **5** shows a new negative band at ~280 nm as well as a positive one at 250 nm. The maximum of the UV absorption curve shifts from 270 nm at room temperature to 265 nm at −195°. Although there are several possible explanations of these results one consistent with the presence of a conformational equilibrium is the following. If λ_{max} for the two conformers occurs at somewhat different wavelengths (differences less than 5 nm) and the signs of the CD bands associated with these bands differ then it is possible to rationalize the low temperature curve. Moscowitz[13a] had shown that when bands of opposite sign overlap, the resulting curve show maxima and minima with relatively large (25–30 nm) separations similar to the low temperature curve of **5**. We made several attempts using curve fitting procedure to define the position, half-bandwidth, etc. of two such bands, however, no satisfactory solutions were found. It is thus not certain that the above description is correct and Legrand[17b] in discussing temperature effects on the sign and magnitude of CD bands, noted that in addition to changes in the ratio of conformers, equilibria between differently solvated species, increased or decreased populations of low energy vibrational species, and aggregates can lead to large and sometimes unexpected changes in the sign and magnitude of CD bands.

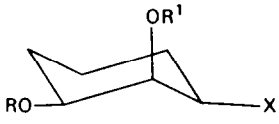
The dihydrodiols, **7**, **9** and **10** obtained from metabolism of a series of *p*-halogenated toluenes by *P. putida*



7 X = CH_3, Y = F
9 X = CH_3, Y = Cl
10 X = CH_3, Y = Br

were isolated and their optical activity examined. Toluene (*p*-H) and *p*-fluorotoluene yielded optically active dihydrodiols, whereas the dihydrodiols **9** and **10** from *p*-chloro- and *p*-bromotoluene were racemic. Although the sign of the CD curve of **7** was identical with that of **1**, the amplitude was very much larger. Hydrogenation of the diene **7** yielded a mixture from which a pure diol was not isolated. Therefore the absolute stereochemistry of **7** is tentatively assigned as identical to that of **1**, although it was not confirmed through use of the "*Dibenzoate Chirality Rule*".[13]

In an earlier study Gibson *et al.*[18] have shown that a mutant strain of *Beijerinckia* oxidizes biphenyl to a *cis*-dihydrodiol, **11**. The diacetate of **11** was hydrogenated to yield **12b**, which was reductively hydrolyzed (LAH) to yield (+)-*cis*,*cis*-**12a** which, by analogy to the toluene



8 (a) X = C_2H_5, R = R^1 = H
(b) X = C_2H_5, R = C(=O)–C_6H_5, R^1 = H
(c) X = C_2H_5, R = R^1 = C(=O)–C_6H_5

12 (a) X = C_6H_5, R = R^1 = H
(b) X = C_6H_5, R = R^1 = C(=O)–CH_3

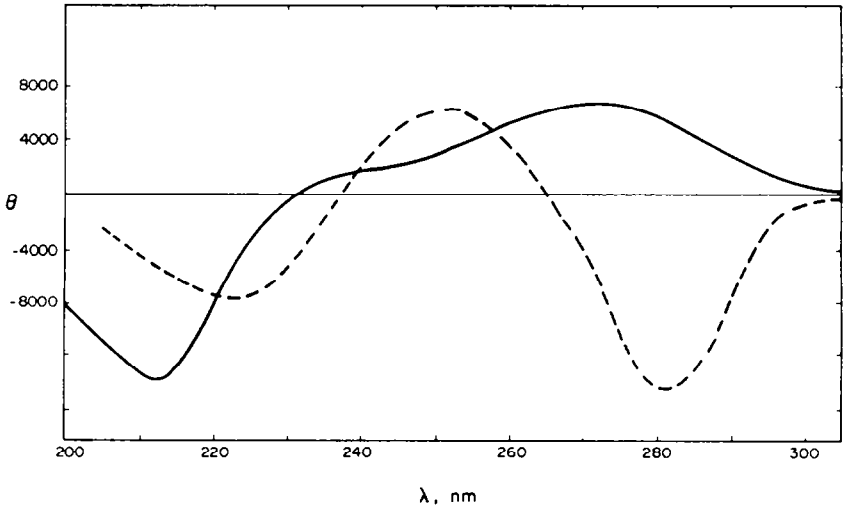


Fig. 1. CD curves of 5 in 1:3 methylcyclohexane/isopentane at room temperature ——, and at ~ −192°C ------.

metabolite, was to be assigned by conversion to optically active 2-phenyladipic acid. Synthetic, optically active (+)-**12a**, prepared by chiral reduction (*l*-darvon LAH) of *cis*-2-acetoxy-6-phenylcyclohexanone,[19] was used for further reactions, as insufficient quantities of the metabolite were available.

Preliminary experiments with racemic **12a** showed that Jones oxidation of this diol yielded a mixture of 2-phenyladipic acid and 4-benzoylbutyric acid. In order to minimize the formation of the latter compound, a two-step oxidative procedure was employed. The diol **12a** was first oxidized with sodium periodate under neutral conditions, and the resulting dialdehyde was oxidized to 2-phenyladipic acid with bromine water in the presence of calcium carbonate. In this procedure, (+)-**12a** yields (+)-2(*S*)-phenyladipic acid.[20] Thus the configurations about the OH bearing carbons for **1** and **11** are identical although the designations of the 2-substituted adipic acids formed on oxidation differ. The difference results from the Prelog–Cahn–Ingold conventions to establish priority.

In a study of the microbiological oxidation (*P. putida*) of (±)-3-methylcyclohexene[21] the two diols formed were separated via their *p*-nitro-benzoates. The diols **2a** and **3a**, obtained by hydrolysis, were converted to their dibenzoates and their CD spectra determined (Discussion). The dibenzoate of **2a** obtained from microbiological oxidation of toluene followed by reduction, and the one obtained from microbiological oxidation of 3-methylcyclohexene were shown to have identical CD curves, and therefore the same absolute stereochemistry. In order to determine the absolute stereochemistry of **3a**, a sample was oxidized with Jones reagent to (+)-2(*S*)-methyladipic acid. The absolute stereochemistry of **3a** can therefore be assigned as *cis*-(1*S*,2*R*)-dihydroxy-3(*S*)-methylcyclohexane.

DISCUSSION

One purpose in examining the optical activity and absolute stereochemistry of **1**, **5**, **6**, **7**, **9**, **10** and **11** is to provide information from which a model describing the oxidation of aromatic hydrocarbons by *Pseudomonas putida* and similar organisms can be developed. The fact that the absolute stereochemistry about the OH-bearing carbons of compounds **1**, **5**, **6**, **7** and **11** is the same suggests that a similar enzyme mechanism operates in both *Pseudomonas* and *Beijerinckia*. The importance of the substituent in orienting the aromatic compound on the enzyme is apparent, since oxidation occurs only *ortho* and *meta* to this substituent. A partial answer to the question of the relative influences of the size of a substituent, compared to its electronegativity, can be obtained from the observation that toluene, ethylbenzene and chlorobenzene yield diols whose absolute stereochemistries about the hydroxyl-bearing carbons are identical. Additional insight into the relative importance of steric and electronic factors was obtained from a study of a series of *p*-halogenated toluene derivatives where, the size of the halogen increases while its electronegativity decreases in the series F, Cl and Br. All the *p*-halogenated toluene derivatives studied were oxidized to *cis*-dihydrodiols; however, only the *p*-fluoro derivative was optically active. As toluene is a good substrate and the resulting dihydrodiol is optically active, it appears that the electronegativity of the *p*-fluorine did not influence the course of the oxidation. The sizes of the other halogen atoms greatly exceed that of hydrogen, and the specificity of the enzyme towards them decreases. The observation that the dihydrodiols from *p*-chloro and *p*-bromo toluene are optically inactive can be rationalized by assuming that the enzyme cannot distinguish among the volumes of a Me, Cl or Br substituent. If the enzyme were sensitive to the polarity of a Cl atom as opposed to a Me substituent, an optically active but not necessarily optically pure product would be expected.

The *cis*-dihydrodiols obtained by microbial oxidation of substituted benzenes and those from naphthalene[2b] and anthracene[22] all have an (*R*) configuration on the carbon adjacent to the substituent and an (*S*) configuration at the more remote carbon.

Although most olefins are presumably oxidized to *trans*-diols, in mammals[3] via epoxide intermediates, we were interested in determining whether *P. putida* 39/D would oxidize an olefin to a *trans*- or *cis*-diol. We chose (±) 3-methylcyclohexene as a substrate and found that both *cis*-diols were formed; no *trans*-diols were observed. The diols **2** and **3** were both optically active and the absolute stereochemistries about the OH-bearing C atoms were identical for the two compounds.

The results of these studies indicate that it is feasible to use bacteria to oxidize a wide variety of aromatic substrates to *cis*-dihydrodiols with some confidence that the absolute stereochemistry about the OH bearing carbons will be (*R*) *ortho* to the substituent and (*S*) *meta*.

EXPERIMENTAL

Microbiological preparation of cis*-dihydrodiols.* Compounds **6**, **7**, **9** and **10** were produced from the correspondingly substituted benzenes by *P. putida*, using the procedure previously described.[5] Each of these dihydrodiols had the proper NMR and mass spectrum for the structure assigned. The NMR spectrum of **6** has been reported[23] and compared to the corresponding *trans* isomer.

(−)cis,cis-1,2-*Dihydroxy*-3-*methylcyclohexane* (**2a**). A soln of 5.5 g of **1**[4] in EtOAc (~50 ml) containing several drops of Et_3N was hydrogenated over 5% Pd/C at atmospheric pressure. The mixture was filtered, concentrated, chromatographed over silica gel with EtOAc:CH_2Cl_2, concentrated, and the crude diol fraction was isolated. This fraction (2.00 g, 15.4 mmol) was dissolved in 10 ml pyridine and 2.23 g (15.8 mmol) of benzoyl chloride was added slowly at 0°–5°. The mixture was allowed to stand overnight at room temp. and worked up by standard procedures to yield a mixture which was purified by chromatography over silica gel (0.2–0.5 mm) with EtOAc:hexane (1:20). The mono-benzoate **2b** (1.821 g) was isolated α_D^{20} −25°(c = 1.05, $CHCl_3$). The NMR spectrum of optically active **2b** was identical with that of authentic racemic material.[8]

The dibenzoate **2c** was prepared by reacting 24 mg of **2b** in 1 ml pyridine with several drops of benzoyl chloride (excess) overnight. The crude dibenzoate was purified by preparative thick-layer chromatography. The 220 MHz NMR spectrum of **2c** (oil) in $CDCl_3$ showed absorption at δ0.99 (d, 3H), δ5.16 (m, 1H), δ5.66 (t, 1H) and complex aromatic absorption (10 H). The sample was identical with one prepared from pure racemic **2a**.[8] The CD spectrum in MeOH showed a negative band at 236 nm (θ = −33,380) and a positive band at 222 nm (θ = +13,350).

A soln of 484 mg of **2b** in 10 ml MeOH containing 5 ml water and 90 mg KOH was refluxed for 1 hr. The solvent was removed *in vacuo*, and the residue extracted with EtOAc, dried, and concentrated to yield an oil **2a** α_D^{20} −37°(c0.97, MeOH), value erroneously reported as −3.7° in Ref. 21. The NMR spectrum of this oil was identical with that of racemic material.

(−)-2(R)-*Methyladipic acid* (**4**). A soln (0.303 g) of **2a** in 5 ml acetone was cooled in an ice-water bath, and Jones reagent[24] was added dropwise until the color persisted for ~5 min. Several drops of isopropyl alcohol was added to decolorize the soln. The acetone soln was decanted, and the Cr^{3+} salts were washed with

acetone. The combined acetone filtrates were concentrated, dissolved in 5 ml isopropanol, and dicyclohexylamine was added until the mixture was alkaline. The soln was concentrated, ether was added and the bis-dicyclohexylamine salt was filtered and washed with ether (yield 255 mg). The salt was recrystallized from isopropanol, m.p. 147–9°, α_D^{20} – 3.0° (c = 3.89, water). It was converted to the corresponding acid by water containing H_2SO_4, and extracted into EtOAc. The 2-methyladipic acid was recrystallized from hexane-ethyl acetate α_D^{20}–14.2° (c4.85, EtOH); reported[6] α_D^{20}–13.4° (EtOH).

Hydrogenation of **5** *and preparation of* **8b** *and* **8c**. A soln of **5** (478 mg) in 25 ml of EtOAc containing several drops of Et_3N was hydrogenated over 5% Pd/C as described for **1**. The crude diol mixture was reacted with ~1 equivalent (537 mg) of benzoyl chloride and worked up in the standard manner. The monobenzoate (**8b**) was isolated by chromatography on silica gel using EtOAc:hexane (1:9) and its stereochemistry assigned as *cis,cis* from its NMR spectrum ($CDCl_3$), δ 4.09 (broadened d, $J_{2,3}$ = 2.3 Hz) and δ 4.98 (1H, octet $J_{1,6}$ = 5 and 11 Hz). The assignments were confirmed by decoupling experiments.

A small sample of **8b** was treated with excess benzoyl chloride in pyridine and worked up in the usual manner. The dibenzoate was purified by thick layer chromatography on silica gel. The NMR spectrum confirmed the assigned structure. The CD curve of **8c** was measured in MeOH and showed a band at 237 nm (θ = – 66,600) and a band at 222 nm (θ = + 30,400).

Diols from microbiological oxidation of (±)-3-*methylcyclohexene*. The crude diol mixture (465 mg) obtained from microbiological oxidation of (±) 3-methylcyclohexene was added to a soln of 1.00 g (1.5 equivs) of *p*-nitrobenzoyl chloride in 6 ml pyridine. The mixture was refluxed for 15 min, poured into dilute aqueous acid, extracted into benzene, washed with $NaHCO_3$aq dried and concentrated. The crude mixture (700 mg) was purified by preparative thick-layer chromatography (2 mm silica gel plates developed with 20:80 EtOAc:hexane) to yield a mixture of the C-1 mono *p*-nitrobenzoate of **2a** and the bis *p*-nitrobenzoate of **3a**. In addition a second more polar zone (174 mg) containing a mixture of the two mono *p*-nitrobenzoates of **3a** was obtained.

A pure sample (115 mg) of the bis *p*-nitrobenzoate of **3a** (m.p. 148–150°; α_D^{20} 126°, c = 1.03, $CHCl_3$) was obtained by crystallizing the mixture from EtOH. The mother liquors from the above crystallization were concentrated and the residue was crystallized from pentane to yield (53 mg) of the C-1 *p*-nitrobenzoate of **2a** (m.p. 87–89°C, α_D^{20} + 35°, c = 1.2, $CHCl_3$). This α_D was erroneously reported as −35° in Ref. 21.

The free diols were obtained by hydrolysis in aqueous methanolic KOH to yield **2a** (α_D^{20}–25°, c = 1.2, $CHCl_3$) and **3a** (α_D^{20} + 35.2°, c = 1.2, $CHCl_3$). The dibenzoates (**2c** and **3c**) were prepared by reacting each diol with 2.5 equivs of benzoyl chloride in pyridine at room temp. overnight. The crude dibenzoates were purified by preparative thick-layer chromatography as described previously. The CD spectrum of **3c** showed bands at 237 nm (θ = + 53,000) and 222 nm (θ = – 17,000).

Oxidation of **3a** *to* (+)2(S)-*methyladipic acid*. The mixture of the two *p*-nitrobenzoates of **3a**, obtained from the more polar zone, was hydrolyzed with KOH in aqueous MeOH to yield **3a** (42 mg). A soln of **3a** (24 mg) in 2 ml of acetone was oxidized with several drops of Jones reagent. The acetone supernatant was removed, concentrated, the residue dissolved in 1 ml isopropanol, and a slight excess of dicyclohexylamine added. The soln was concentrated, ether added to ppt the *bis*-dicyclohexylamine salt (36 mg, m.p. 140–143°C).

The salt (15 mg) was dissolved in 3 ml EtOH which was approximately 1N H_2SO_4. The filtered soln, in a 1 cm cell, had an observed rotation of 0.004° at 436 nm (α_{436}^{20} + 29°) using a Cary 60 spectropolarimeter. A sample of authentic (−)-2(*R*)-methyladipic acid prepared by oxidation of **1a** had an α_D^{20} of −29° under the same conditions.

Acetylation and hydrogenation of **11**. To a soln of **11** (160 mg) in 1 ml pyridine was added Ac_2O (3 ml) and the soln allowed to stand overnight at room temp. The product was worked up as usual and purified by preparative thick layer chromatography on silica gel using chloroform to yield 154 mg (oil) of the diacetate, α_D^{25}–51.6° (c = 7.7, EtOAc). The diacetate was hydrogenated in EtOAc (10 ml) containing one drop of Et_3N over 40 mg of 5% Pd/C to yield 134 mg of a mixture of cis,cis and cis,*trans* 1,2-*diacetoxy*-3-*phenylcyclohexane*.

Preparation and reduction of cis,cis 1,2-*diacetoxy*-3-*phenylcyclohexane* (**12b**). The diacetate (56 mg) was treated with excess LAH in ether at 0° for 3 hr. After decomposing unreacted hydride with water, 2N H_2SO_4 was added to dissolve the aluminum hydroxide. The mixture was extracted with EtOAc and the extract was washed successively with $NaHCO_3$aq and water, then dried over Na_2SO_4. The solvent was removed to give a slightly yellow solid (35 mg). This was purified by thick-layer chromatography (silica gel, 2 mm thickness, 1:1 EtOAc-hexane) and the more polar band was collected to give 30 mg of **12a** as crystals (m.p. 70–79°). Repeated recrystallization from benzene-hexane gave 12 mg of colorless needles, m.p. 85–86°, α_D^{20} + 72.7° (c = 0.605, MeOH).

Oxidation of (+) cis,cis-3-*phenylcyclohexan*-1,2-*diol* (*prepared by asymmetric synthesis*) (**12a**). To a soln of sodium periodate (37 mg) in 70% aqueous EtOH (3 ml) was added **12a**[25] (20 mg, α_D^{25} + 33.8°, c = 2.16, MeOH) in the same solvent at 0°. The mixture was stirred at 0° for 3 hr, then diluted with water and extracted with EtOAc. The extract was washed with satd NaClaq and dried over Na_2SO_4. Removal of solvent under reduced pressure gave 14 mg 2-phenyladipic dialdehyde as colorless oil which was submitted to further oxidation without purification.

To a cold soln of the dialdehyde in 10 ml water, containing $CaCO_3$ (60 mg), Br-water (1.2 ml), prepared from 0.5 ml of Br_2 and 25 ml water, was added with stirring. Stirring was continued for 3 hr at 0°, and the soln acidified with a few drops of conc HCl. The mixture was extracted with EtOAc, and the extract was washed with satd $NaHCO_3$aq. The alkaline layer was washed with ether, acidified with a few drops of conc HCl and then extracted with EtOAc. The extract was dried over Na_2SO_4; removal of solvent yielded 11 mg of (+)-2(*S*)phenyladipic acid as colorless crystals.[20]

The NMR spectrum of optically active (+)-2(*S*)-phenyladipic acid [α_D^{25} + 26.9° (c = 0.55 EtOH), optical purity 42% based on the absolute rotation in the literature] was identical with that of authentic racemic material prepared by oxidation of racemic *cis,cis*-3-phenylcyclohexan-1,2-diol in an analogous manner.

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