Communications to the Editor

Enantiomeric Excess and Absolute Configuration Determination of *cis*-Dihydrodiols from Bacterial Metabolism of Monocyclic Arenes

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We report a new and generally applicable method for the determination of enantiometic excess (ee) and absolute configuration of a series of *cis*-dihydrodiol metabolites of monosubstituted arenes obtained from growing cultures of a mutant strain of the bacterium *Pseudomonas putida* UV4. The formation of cycloadducts with 4-phenyl-1,2,4-triazoline-3,5-dione and conversion to single crystalline di-MTPA diastereoisomers allows stereochemical analysis by X-ray crystallography and ^1H and ^{19}F NMR spectroscopy.

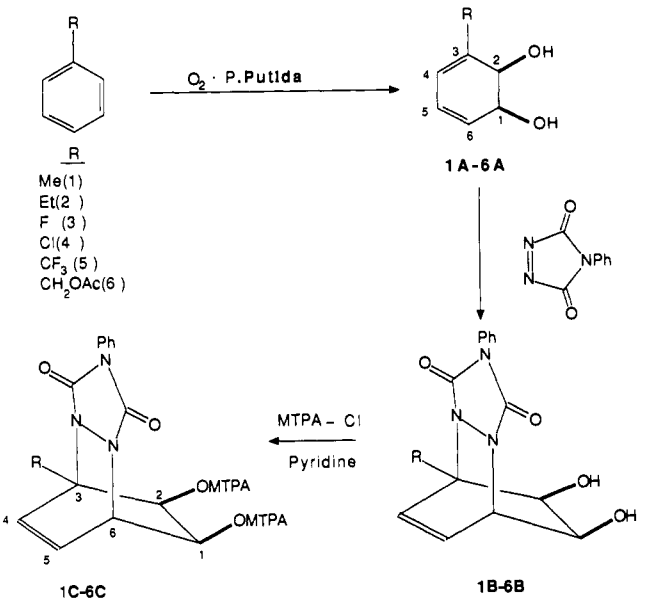
Although a considerable number of *cis*-dihydrodiols (>120) resulting from bacterial metabolism of arenes have been reported in the literature, a relatively small proportion (<10%) are of known optical purity or absolute stereochemistry. Included among those stereochemically assigned are metabolites of monocyclic (e.g., toluene,[1] ethylbenzene,[2] and chlorobenzene[3-5]) and polycyclic (e.g., naphthalene,[6] anthracene,[7] phenanthrene,[8] and benz[*a*]anthracene[9]) arenes where a range of methods including stereochemical correlation and CD spectroscopy were used. The requirement for a generally applicable method for optical purity and absolute configuration determination has markedly increased with the commercial availability of *cis*-dihydrodiols as chiral synthons and their recent use in synthesis.[4,5,10-19]

Table I. Optical Rotations, Enantiomeric Excess Values, and Absolute Configurations

compd[a]	$[\alpha]_D$[b]	compd	$[\alpha]_D$[b]	% ee[c]	config
$\mathbf{1C}_R$	−20	**1A**	+72	>98	1*S*,2*R*
$\mathbf{1C}_S$	+23		(+26)[d]		
$\mathbf{2C}_R$	−29	**2A**	+43	>98	1*S*,2*R*
$\mathbf{2C}_S$	+29		(+40)[d]		
$\mathbf{3C}_R$	+10	**3A**	−33	ca. 60	1*S*,2*S*[e]
$\mathbf{3C}_S$	+23				
$\mathbf{4C}_R$	+2	**4A**	+36	>98	1*S*,2*S*[e]
$\mathbf{4C}_S$	+36				
$\mathbf{5C}_R$	−11	**5A**	−63	>98	1*S*,2*R*
$\mathbf{5C}_S$	+16				
$\mathbf{6C}_R$	+18	**6A**	+39	>98	1*S*,2*R*
$\mathbf{6C}_S$	+16				

[a] Subscripts *R* and *S* refer to the configuration of the MTPA used in esterification. [b] Degrees in $CHCl_3$ solvent. [c] Enantiomeric excess determined by ^1H NMR analysis of the MeO signals of **1C-6C**. [d] Degrees in MeOH solvent. [e] The apparent reversal of configuration at C-2 is due to the sequence rule.

In the present study the *cis*-dihydrodiol metabolites of toluene (**1A**), ethylbenzene (**2A**), fluorobenzene (**3A**), chlorobenzene (**4A**),



(trifluoromethyl)benzene (**5A**), and benzyl acetate (**6A**) were isolated as biotransformation products from *P. putida* UV4 (Table I). *cis*-Dihydrodiols **1A**,[1] **2A**,[2] **4A**,[3-5] **5A**,[20,21] and **6A**[18] were

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previously obtained as bacterial metabolites of the parent arenes by *P. putida*, but in many cases, $[\alpha]_D$ values, ee values, and absolute configurations (**3A**, **5A**, **6A**) were unreported.

The chiral lanthanide shift reagent Eu(hfc)$_3$ has proved to be very effective in the determination of ee values of a range of diols,[22] including the dihydrodiols of polycyclic arenes and azarenes (e.g., naphthalene and quinoline). By this method distinguishable ^{1}H NMR signals were found for each enantiomer.[23] When a chemically synthesized racemic sample of the monocyclic *cis*-dihydrodiol **1A** and an enzymatically formed sample of the *cis*-dihydrodiol **3A** of low optical purity (table) were similarly analyzed,[23] the ^{1}H NMR signals for enantiomers were found to be indistinguishable at all concentrations of Eu(hfc)$_3$. The present report illustrates how this problem can be circumvented in the *cis*-dihydrodiol series by synthesis of appropriate diesters from α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA).

Initial attempts to form di-MTPA esters of the *cis*-dihydrodiols **1A–6A** resulted in their total aromatization. *cis*-Diols **1A–6A** were, however, found to react with 4-phenyl-1,2,4-triazoline-3,5-dione at ambient temperature (12 h) to give cycloadducts **1B–6B**. ^{1}H NMR analysis of the crude samples of **1B–6B** showed essentially a single diastereoisomer (>97%) to be present. Pure diastereoisomers **1B–6B** were obtained by PLC purification in ca. 70–80% yield. Treatment of the cycloadducts **1B–6B** with the acid chloride of (+)-(*R*)-MTPA in pyridine yielded the corresponding di-MTPA esters (**1C**$_R$–**6C**$_R$) in quantitative yield (table).

The *cis*-dihydrodiol metabolite **1A** ($[\alpha]_D$ +25.6°, MeOH) had previously been shown to be optically pure and of the 1*S*,2*R* configuration and thus the di-MTPA ester **1C**$_R$ has the *R*′,*R*″,1*S*,2*R* configuration (*R*′ and *R*″ refer to the absolute configurations of the MTPA groups in the di-MTPA ester.). Since the 1*R*,2*S* enantiomer of *cis*-dihydrodiol **1A** was unavailable in sufficient quantities either as a metabolite or by synthesis,[23] it was impossible to synthesize the corresponding (*R*′,*R*″,1*R*,2*S*)-di-MTPA diastereoisomer in order to observe the characteristic δ values in the ^{1}H NMR spectrum that might distinguish it from the **1C**$_R$ configuration. However, diesterification of the cycloadduct **1B** derived from the 1*S*,2*R* enantiomer of *cis*-dihydrodiol **1A** with the acid chloride of (−)-(*S*)-MTPA yielded the di-MTPA ester of *S*′,*S*″,1*S*,2*R* configuration (**1C**$_S$), which is the enantiomer of the elusive *R*′,*R*″,1*R*,2*S* diastereoisomer and hence spectrally indistinguishable from it. The diagnostic δ values for diastereoisomers **1C**$_R$ and **1C**$_S$ are thus identical with those that could be found for the di-MTPA esters derived from each enantiomer of **1A** by using a single enantiomer of MTPA. Use of both the respective acid chlorides of (+)-(*R*)- and(−)-(*S*)-MTPA in the diesterification of the cycloadducts of *cis*-dihydrodiols **2A**, **4A**, **5A**, and **6A**, again showed each sample to be homochiral. The distinguishable MeO ($\delta_{^1H}$) and CF$_3$ ($\delta_{^{19}F}$) signals in the NMR spectrum of each di-MTPA diastereoisomer obtained from the cycloadduct **3B** of *cis*-diol **3A** with the acid chloride from either (+)-(*R*)-or (−)-(*S*)-MTPA allowed a value of ca. 60% ee to be estimated (table). Fractional recrystallization of **3B** yielded the major enantiomer in pure form. It has also proved possible to use this method in the determination of optical purity of the *cis*-dihydrodiols of naphthalene and quinoline after catalytic hydrogenation to yield *cis*-tetrahydrodiols followed by diesterification using both (+)-*R* and (−)-*S* forms of MTPA.[23]

X-ray crystal structure analysis was carried out on suitable crystals of compounds **1C**$_S$, **3C**$_R$, and **5C**$_S$, and it revealed that the cycloaddition reaction between the *cis*-dihydrodiols **1A**, **3A**, and **5A** and the triazolinedione dienophile had occurred almost exclusively (>97%) syn to the hydroxyl groups (yielding essentially the single diastereoisomers **1B**, **3B**, and **5B**). Previous cycloadditions on the diester or acetonide derivatives of *cis*-dihydrodiols (of structure similar to compounds **1A**, **3A**, and **5A**) using a range of dienophiles often yielded two or four diastereoisomers from each enantiomer, i.e., exo and endo isomers of both anti[21,24-27] and syn[21,27] cycloadducts.

Since the absolute configuration of the MTPA group for the diesters **1C**, **3C**, and **5C** is known, the configurations of the other chiral centers and of the parent *cis*-dihydrodiols **1A**, **3A**, and **5A** can be unequivocally established (table). The 1*S*,2*R* configuration is established for metabolite **5A** and is also confirmed for metabolite **1A**. The major enantiomer found in the metabolite **3A** is now assigned a 1*S*,2*S* configuration.

The absolute configuration of each *cis*-dihydrodiol enantiomer [now firmly established by X-ray crystallographic (**1A**, **3A**, **5A**) and other[1-5] methods (**1A**, **2A**, **4A**)] was associated with several diagnostic ^{1}H and ^{19}F NMR signals (500 MHz, $CDCl_3$) in the respective di-MTPA esters (**1C–6C**). Thus, a *cis*-dihydrodiol having a 1*S* configuration gave a di-MTPA ester that was characterized by (i) the downfield MeO signal having a larger positive $\delta_{^1H}$ value (3.61–3.65 ppm) and (ii) the downfield CF$_3$ signal having a smaller negative $\delta_{^{19}F}$ value (−7.66 to −8.39 ppm) when the (+)-*R* enantiomer of MTPA was used. Conversely, the di-MTPA ester from a *cis*-dihydrodiol having a 1*R* configuration shows a smaller δ_1 value (3.21–3.55 ppm) and a larger negative δ_{19} value (−8.72 to −9.28 ppm) for the corresponding signals when (+)-(*R*)-MTPA is utilized.

The applicability of this method to *cis*-dihydrodiols of monocyclic arenes bearing several substituents is currently under investigation.

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Synthesis of the First 2,2′-Biphosphinine. X-ray Crystal Structure Analysis of Its Tetracarbonylchromium Complex

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2,2′-Bipyridines have found widespread use in analytical and synthetic coordination chemistry over many decades.[1] Complexes such as $[Ru(bipy)_3]^{2+}$ have special redox and photocatalytic properties[2] which have been the subject of extensive studies. In view of that situation, the design of phosphorus analogues of bipyridines was an attractive goal. Some time ago, we synthesized a monophosphorus analogue **1**[3] and performed a preliminary investigation of its coordinating properties.[4]

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