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Stereoselective *cis*-dihydroxylation of azulene and related non-aromatic polyenes

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Abstract

Dioxygenase-catalysed dihydroxylation of azulene and related non-aromatic polyenes has been found to yield enantiopure chiral *cis*-diols of synthetic potential.

Highly functionalised enantiopure compounds containing carbocyclic rings of differing sizes are useful synthons for natural product synthesis. Although there have been many reports on the isolation and application of *cis*-dihydrodiol metabolites of substituted benzenes, over the past decade,[1] very little work has been reported on the metabolism of non-aromatic polyenes. In this paper, we report the biotransformation of azulene and conjugated monocyclic polyenes, containing 5–8 carbon atoms, to produce metabolites for use in future synthetic studies.

cis-Dihydroxylation of the bicyclic arene naphthalene, **1**, to yield the corresponding *cis*-dihydrodiol enantiomer, **2**, has been reported using dioxygenase enzymes.[2–4] A similar *cis*-dihydroxylation of the isoelectronic bicyclic hydrocarbon azulene, **3**, has not been previously observed. The present study of azulene, **3**, and a range of related dienes **12**, **14**, **16**, **18** and triene substrates **7** and **10** of differing ring sizes is part of a wider programme to assess the value of dioxygenase enzymes as biocatalysts for oxidation reactions of non-aromatic substrates.[5,6]

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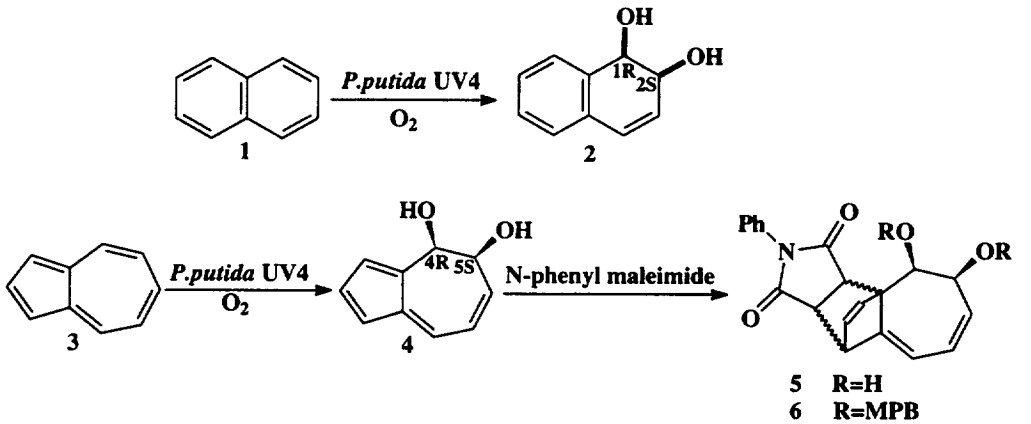


Virtually all examples of *cis*-dihydroxylation of alkenes, reported using dioxygenases, were aryl substituted alkenes.[5–7] Evidence of *cis*-dihydroxylation and/or allylic monohydroxylation was initially sought using whole cell preparations of the bacterium *Pseudomonas putida* UV4, a source of toluene dioxygenase (TDO), and polyene substrates.

Azulene, **3**, having less aromatic character (resonance energy ca. 45 kcal mol^{-1}) than naphthalene **1** (resonance energy ca. 61 kcal mol^{-1}), also has more options for regioselective *cis*-dihydroxylation. Thus, while naphthalene, **1**, yielded a single *cis*-dihydrodiol bioproduct, **2**, azulene, **3**, could, in principle, yield three *cis*-diols due to dihydroxylation at the 1,2-, 4,5-, or 6,7-bonds.

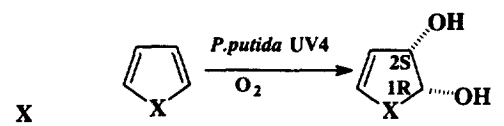
Biotransformation of azulene, **3**, using intact cells of *P. putida* UV4 under reported conditions[8] was found to be regioselective and gave a single metabolite (ca. 20% isolated yield). Based upon chromatographic (TLC, PLC, HPLC), spectral (^{1}H-COSY, NOE and ^{13}C-NMR, IR, MS), microanalytical, and chiroptical ($[\alpha]_D^{25}$ −107, MeOH) data the bioproduct was identified as the *cis*-diol **4**. Metabolite **4** proved to be relatively stable in neutral aqueous solution. *cis*-Dihydrodiol **2**, obtained from naphthalene, **1**, was found to decompose under acidic conditions at a faster rate (×6) compared with *cis*-diol metabolite **4**. Purification by PLC (silica gel) and recrystallisation yielded compound **4** as a pure product which slowly decomposed in air at ambient temperature to yield an intractable polymeric material. This behaviour is consistent with a fulvene structure. Further confirmation of the *cis*-diol diene (fulvene) structure of compound **4**, was obtained by formation of a mixture of stable stereoisomeric cycloadducts **5** when N-phenyl maleimide was used as a dienophile. The mixture of cycloadducts was separated by preparative HPLC (Phenomenex Primesphere 5 C-18, MeOH/$H_2$0). Conversion of the major cycloadduct, separated from the diastereomeric mixture **5**, to the corresponding cyclic boronate derivatives, **6**, formed using (+)-(*R*)- and (−)-(*S*)-2-(1-methoxyethyl)-phenyl boronic acid (MPBA),[9] indicated that the enantiomeric excess (*ee*) value of the *cis*-diol metabolite **4** was ≥98%. The novelty of this oxidation lies in the concomitant loss of aromaticity in both rings. Previous dioxygenase-catalysed *cis*-dihydroxylations of polycyclic arenes, e.g. naphthalene, **1** involved only the loss of aromaticity in one ring.

Azulene **3** may be considered as two cyclic sub-units, i.e. an electron deficient cyclohexatriene ring and an electron rich cyclopentadiene or fulvene ring. It is noteworthy that TDO-catalysed *cis*-dihydroxylation of **3** occurs exclusively at the more electron deficient 4,5-double bond and ring (cycloheptatriene).

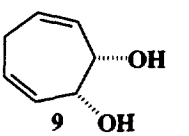
Table 1
Chiroptical data for *cis*-diols **8**, **11**, **13**, **15**, **17** and **19**

Compound No.	*ee* [a]	$[\alpha]_D^{25}$	Abs. Config.	Lit. [10] *ee*	Lit. [10] $[\alpha]_D^{25}$
8	>98%	+21, c 0.5 ,$CHCl_3$	(1R,2S)	24%	+6
11	>98%	+89, c 0.5, EtOH	(1R,2S)	30%	+28
13	20% [b]	+13, c 0.4, $CHCl_3$	(1R,2S)	38%	+26
15	>98%	+107, c 0.5, $CHCl_3$	(1R,2S)	37%	+39
17	>98%	+59, c 0.6, $CHCl_3$	(1R,2S)	21%	+13
19	>98%	-19, c 0.7, $CHCl_3$	(1R,2S)	21%	+1

[a] Determined by formation of diMTPA esters using both (R)- and (S)- enantiomers of methoxy (trifluoromethyl) phenylacetic acid (MTPA). [b] Using the MPBA method. [9]



X		
$CH{=}CHCH_2$	7	8
$C{=}CMe_2$	10	11
CH_2	12	13
$(CH_2)_2$	14	15
$(CH_2)_3$	16	17
$(CH_2)_4$	18	19



In order to gain further information on the regioselectivity and facial selectivity of TDO-catalysed *cis*-dihydroxylation of azulene, **3**, the related conjugated triene structures cycloheptatriene, **7**, and dimethyl fulvene, **10**, and also the conjugated cyclic dienes, **12**, **14**, **16** and **18**, were added as substrates to cultures of *P. putida* UV4. The isolated yields (ca. 30%) of chiral *cis*-diol bioproducts have not been optimised. The specific rotations $[\alpha]_D^{25}$, and *ee* values obtained are listed and compared with the literature $[\alpha]_D^{25}$ values using identical solvents[10] in Table 1.

TDO-catalysed *cis*-dihydroxylation of cycloheptatriene, **7**, yielded a mixture of chiral diol **8** and achiral diol **9** in the ratio 2:1. The *cis*-dihydrodiol derivative **13** from cyclopentadiene **12** was an exception in this series in having a lower *ee* value (20%). The absolute configurations of the *cis*-diol enantiomers **8**, **11**, **13**, **15** and **17**, synthesised by the Sharpless asymmetric dihydroxylation method[10] (AD-mix-β) or obtained by the TDO-catalysed method were identical (1*R*,2*S*) based upon the reported[10] absolute configurations. Enantiomeric excesses of *cis*-diols obtained using TDO (**4**, **8**, **11**, **15**, **17** and **19**) were ≥98%, while those synthesised using AD-mix-β[10] (**8**, **11**, **13**, **15**, **17** and **19**) were in the range 5–40%. When the naphthalene dioxygenase (NDO) enzyme system, present in the *P. putida* NCIMB 8859 and 9816/11 strains, was used as a biocatalyst with azulene **3** or cyclopentadiene **12**, no *cis*-diol product was isolated. However using the NDO system with substrates **7**, **10**, **14**, **16** and **18** gave the corresponding *cis*-dihydrodiols **8**, **11**, **15**, **17** and **19** of identical *ee* values (≥98%) and absolute configurations, but having lower yields compared to those obtained using TDO. The NDO biocatalyst, present in *P. putida* strain NCIMB 9816/11, was found to be more regioselective than the TDO strain when cycloheptatriene **7** was used as a substrate; it yielded only the chiral *cis*-diol **8**. Based on the (1*R*,2*S*) configuration, assigned to the *cis*-diol bioproducts **8**, **11**, **13**, **15** and **17**, the configurations of the other metabolites **4** and **19** are assumed to be analogous, i.e. (4*R*,5*S*) and (1*R*,2*S*) respectively.

No evidence of TDO-catalysed monohydroxylation was observed at the allylic methylene groups in di-

enes **12**, **14**, **16** and **18** or triene **7**. Similarly when triene **10** was used as substrate only *cis*-dihydroxylation was observed. Low yields (<5%) of both allylic hydroxylation and alkene *cis*-dihydroxylation products were recorded with cyclohexene substrates using the TDO system.[7,11] Preliminary biotransformation data, obtained using acyclic diene substrates (methyl and ethyl substituted butadienes), has confirmed that dioxygenase-catalysed dihydroxylation also occurs in acyclic polyene substrates. ^{1}H-NMR and GC–MS analyses indicate that the TDO system catalyses dihydroxylation in a regioselective manner, e.g. monosubstituted alkenes being preferred over trisubstituted alkenes and *trans*-disubstituted being the least attractive type of alkene substrate. The volatility of the butadiene substrates resulted in lower isolated yields.

In summary, the present findings, allied to the recent report of the formation of *cis*-diol **11** of unspecified enantiopurity and absolute configuration from the biotransformation of triene **10** using isopropylbenzene 2,3-dioxygenase,[12] suggest that dioxygenase enzymes of different types can catalyse the *cis*-dihydroxylation of cyclic as well as acyclic polyene substrates. The biotransformation route to *cis*-diol derivatives of polyenes offers, in terms of stereoselectivity and regioselectivity, advantages over currently available catalytic asymmetric osmylation methods. The availability of enantiopure *cis*-diol bioproducts **4**, **8**, **11**, **15**, **17** and **19** in a single enzyme-catalysed step now allows their potential as chiral precursors in synthesis to be explored and synthetic studies to this end are currently in progress in our laboratories.

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