Tetrahedron Letters, Vol. 33, No. 9, pp. 1241-1244, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press plc

A ^{1}H-NMR METHOD FOR THE DETERMINATION OF ENANTIOMERIC EXCESS AND ABSOLUTE CONFIGURATION OF *CIS*-DIHYDRODIOL METABOLITES OF POLYCYCLIC ARENES AND HETEROARENES

Derek R. Boyd,*[a] Narain D. Sharma,[a] Rosemary Boyle,[a] R. Austin S. McMordie,[a] Jagdeep Chima,[b] and Howard Dalton.*[b]

[a]School of Chemistry, The Queen's University of Belfast, Belfast BT9 5AG, U.K.

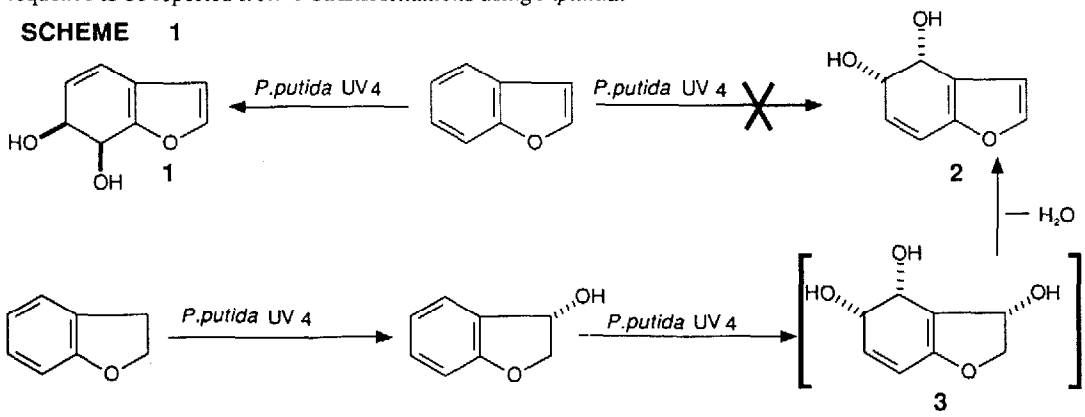
[b]Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, U.K.

Keywords: Bacterial oxidation; *Pseudomonas putida* UV4; *cis*-dihydrodiol stereochemistry; benzofuran.

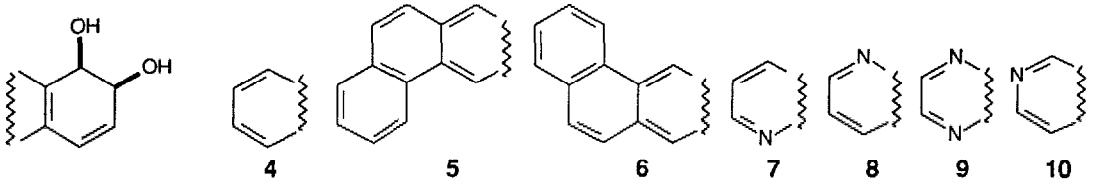
Abstract: Metabolism of benzofuran and 2,3-dihydrobenzofuran by *P.putida* UV4 each yielded *cis*-dihydrodiols; catalytic hydrogenation of the *cis*-dihydrodiols obtained from polycyclic arenes and azaarenes followed by diMTPA ester formation (using both R- and S-MTPA) on the *cis*-tetrahydrodiols formed the basis of a ^{1}H-NMR method for the determination of both enantiomeric excess and absolute configuration.

Metabolism of polycyclic aromatic hydrocarbons (e.g. naphthalene[1,2], anthracene[3,4], phenanthrene[3,5], benz(a)anthracene[6,7] and benzo(a)pyrene[6]) and aromatic heterocycles (e.g. quinoline[8], isoquinoline[8], quinoxaline[8], quinazoline[8], dibenzofuran[9] and dibenzothiophene[10]) by bacteria (*Pseudomonas putida* or a *Beijerinckia* species) has resulted in the formation of *cis*-dihydrodiol bioproducts. As part of a continuing biotransformation programme using heterocyclic substrates and a mutant strain (UV4) of the bacterium *P.putida* , the *cis*-dihydrodiol **1** has been isolated as a new major metabolite of benzofuran without any evidence for the formation of the isomeric *cis*-dihydrodiol **2**. The latter bioproduct was, however, isolated after addition of either 2,3-dihydrobenzofuran or 3-hydroxy-2,3-dihydrobenzofuran to growing cultures of the same bacterium. Formation of *cis*-dihydrodiol **2** from 2,3-dihydrobenzofuran is consistent with a sequence involving benzylic hydroxylation to form intially, 3-hydroxy-2,3-dihydrobenzofuran (an arene hydrate of benzofuran), followed by *cis*-diol formation at the 4,5-bond to yield a transient triol intermediate **3** which undergoes rapid dehydration (Scheme 1). A similar rationalization has previously[11] been applied to the formation of *cis*-dihydrodiol **4** when 1,2-dihydronaphthalene was metabolized. The present observation is thus the second example of a monol ---> triol ---> diol metabolic sequence to be reported from biotransformations using *P.putida*.

SCHEME 1



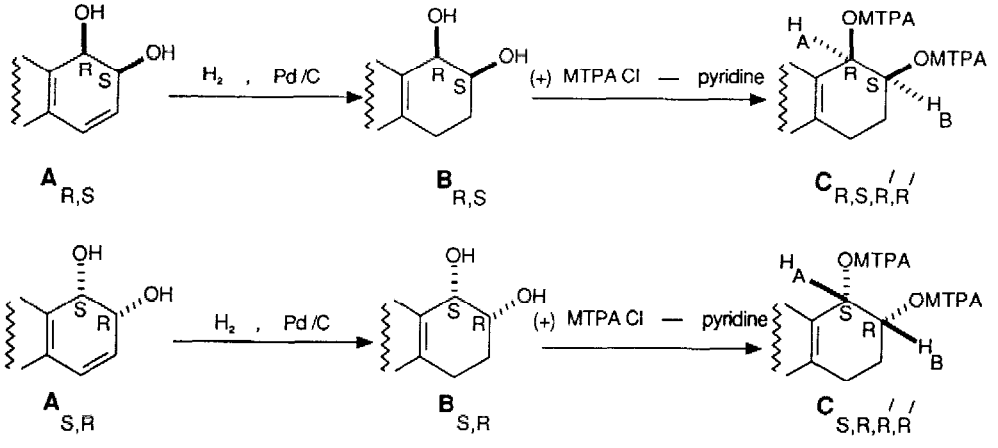
In contrast with the *cis*-dihydrodiol metabolites of PAHs[1-7], where absolute configurations and optical purity values were generally obtainable by stereochemical correlation methods, very few stereochemical analyses have been carried out on *cis*-dihydrodiol metabolites of heterocycles. The isolation of *cis*-dihydrodiol derivatives of benzofuran (**1** and **2**) during the present study, allied to earlier work on the biotransformation of heterocycles by *P.putida*,[8] prompted the quest for a widely applicable and convenient method for the determination of enantiomeric excess (e.e.) and absolute configuration. A general method based on ^{1}H-NMR spectral analysis has now been applied to the *cis*-dihydrodiols of PAHs (**4**, **5**, **6**) and hetero-arenes (**1**, **2**, **7**, **8**, **9**) (Table 1).



4 **5** **6** **7** **8** **9** **10**

We have demonstrated the value of di-α-methoxy-(α-trifluoromethyl)phenylacetic acid (diMTPA) esters of stable 4-phenyl-1,2,4-triazoline-3,5-dione cycloadducts in the stereochemical analysis (e.e. and absolute configuration) of *cis*-dihydrodiol metabolites of substituted benzene substrates from *P.putida*. [12] Attempts to form the diMTPA esters of *cis*-dihydrodiols directly, in both the substituted benzene series[12] and in the bicyclic series (**1,2,4-10**) generally resulted in aromatization of the dihydrodiols. Catalytic hydrogenation of *cis*-dihydrodiols (**A**) (Pd/C,H_2, MeOH or EtOAc, r.t.) provided the more stable *cis*-tetrahydrodiols (**B**) (Scheme 2). With the exception of the *cis*-tetrahydrodiol from compound **10** (which decomposed during attempted diMTPA ester formation) the *cis*-tetrahydrodiols **1**, **2**, **4-9**, (**B**) upon treatment with the appropriate enantiomer of MTPA-chloride in pyridine (16h, r.t.) all yielded the corresponding diMTPA esters **C** in good yield (> 90%).

SCHEME 2



Scheme 2 illustrates the sterochemical relationship between a single enantiomer of *cis*-dihydrodiol ($\mathbf{A}_{R,S}$ or $\mathbf{A}_{S,R}$), the corresponding *cis*-tetrahydrodiol ($\mathbf{B}_{R,S}$ or $\mathbf{B}_{S,R}$) and the diMTPA diastereoisomer formed using (R)-MTPA ($\mathbf{C}_{R,S,R,R'}$, or $\mathbf{C}_{S,R,R',R'}$). The diastereoisomeric excess of the diMTPA esters (**C**), which provides an indirect measure of the enantiomeric excess of the original *cis*-dihydrodiol metabolite (**A**), was readily determined from the MeO singlets in the ^{1}H-NMR spectrum. Thus, the diMTPA esters derived from naphthalene (**4C**), benz (a)anthracene (**5C,6C**) and quinoline (**7C,8C**) (where both enantiomers were available by chemical synthesis[13])

Table 1. ^{1}H-NMR spectral data for the *cis*-tetrahydrodiol diMTPA esters (C); optical rotation, enantiomeric excess, and absolute configuration of the *cis*-dihydrodiols (A)

cis-Tetrahydrodiol diMTPA Esters			*cis*-Dihydrodiol			
Compound	^{1}H-NMR data[a]			$[\alpha]_D^o$	% e.e.[b]	Absolute
	δ_{H_A}	$\delta_{H_A} - \delta_{H_B}$				Configuration
4C[c]	6.33	0.79	**4A**$_{R,S}$	+246	> 98	1R, 2S
4C[d]	6.47	0.94		($CHCl_3$)		
5C[d]	6.56	0.99	**5A**$_{S,R}$[e]	-37[e]	> 98[e]	8S, 9R[e]
5C[c]	6.44	0.85		(THF)		
6C[c]	6.62	0.95	**6A**$_{R,S}$	+361	> 98	11R, 10S
6C[d]	6.76	1.07		(THF)		
7C[c]	6.30	0.73	**7A**$_{R,S}$	+220	> 98	5R, 6S[f]
7C[d]	6.43	0.83		(THF)		
8C[c]	6.56	0.90	**8A**$_{R,S}$	+45	> 98	8R, 7S[f]
8C[d]	6.57	0.94		(MeOH)		
9C[c]	6.56	0.82	**9A**$_{R,S}$	+210	> 98	5R, 6S[f]
9C[g]	6.56[g]	0.88[g]		(MeOH)		
1C[h,i]	6.37	0.86	**1A**$_{S^i,S}$	-35	> 98	7S,[i] 6S[f]
1C[j,i]	6.47[j,i]	0.96[j,i]		(MeOH)		
2C[c]	6.03	0.58	**2A**$_{R,S}$	+16[k]	*ca.*85	4R, 5S[f]
2C[g]	6.23[g]	0.78[g]		(MeOH)		

[a]$CDCl_3$ solvent; [b]determined from the relative peak areas of OMe signals in each diastereoisomer (C); [c]R,S,R',R' isomer; [d]S,R,R',R' isomer; [e]chemically synthesised sample of the opposite configuration to the bacterial metabolite; [f]previously unreported absolute configuration; [g]obtained using the R,S,S',S' isomer which is equivalent to the S,R,R',R' isomer in the ^{1}H-NMR spectrum; [h]S,S,R',R' isomer; [i]due to the Sequence Rule priorities the configuration at C-7 is [S] and thus the trend observed is still consistent; [j]obtained using the S,S,S',S' isomer which is equivalent to the R,R,R',R' isomer; [k]minimal value due to compound instability.

showed discrete MeO signals for both diastereoisomers in each case. This readily confirmed that the *cis*-dihydrodiol metabolites **4**, **5**, **6**, **7** and **8** were enantiomerically homogeneous (> 98% e.e.).

The *cis*-dihydrodiol metabolite **2** obtained from 2,3-dihydrobenzofuran was, by the latter method, found to be *ca.* 85% e.e. The total absence of the MeO-signals corresponding to one of the two possible diastereoisomers of **1C** and **9C** was consistent with e.e. values of > 98%. Whether or not the diastereoisomer **1C**$_{R,S,R',R'}$ (or **9C**$_{R,S,R',R'}$) (obtained from R-MTPA) could be unequivocally distinguished from the **1C**$_{S,R,R',R'}$ (or **9C**$_{S,R,R',R'}$) diastereoisomer by ^{1}H-NMR spectroscopy was investigated. Use of (S)-MTPA allowed the synthesis of **1C**$_{R,S,S',S'}$ (or **9C**$_{R,S,S',S'}$) diastereoisomers from the corresponding *cis*-dihydrodiol metabolites which are spectrally indistinguishable from the **1C**$_{S,R,R'R'}$ (or **9C**$_{S,R,R',R'}$) diastereoisomers but readily

distinguishable from the $\mathbf{1C}_{R,S,R',R'}$ (or $\mathbf{9C}_{R,S,R',R'}$) diastereoisomers on the basis of MeO and other signals. Since the diMTPA diastereoisomers (**10C**) could not be synthesised, the optical purity of metabolite **10A** ([α]-2.5°)[8] was determined (< 5% e.e.) by using the chiral lanthanide shift reagent method ($Eu(hfc)_3$/ CD_3CN) which rendered the allylic proton non-equivalent.

The absolute configurations of the *cis*-dihydrodiol metabolites of naphthalene (**4**),[2] benz(a)anthracene (**5, 6**)[7], quinoline (**7, 8**)[13], quinoxaline (**9**)[13] and benzofuran (**1**)[13] have been unequivocally assigned by stereochemical correlation methods. For these *cis*-dihydrodiol metabolites, the R-configuration at the benzylic centre was associated with a smaller difference in δ values for protons H_A and H_B ($\delta_{H_A} - \delta_{H_B}$) as shown in Table 1. The diMTPA esters of *cis*-tetrahydrodiols **4B, 5B, 6B, 7B** and **8B** having an S benzylic configuration were found to have a larger difference in δ values ($\delta_{H_A} - \delta_{H_B}$). Where benzylic proton signals were distinguishable, it was observed that the values (δ_{H_A}) were smaller for the benzylic R configuration. This consistent trend allowed the *cis*-dihydrodiol **2** to be tentatively assigned the absolute configuration shown (Table 1).

From analysis of the ^{1}H-NMR data (Table 1), it is evident that (a) the *cis*-dihydrodiols of polycyclic arenes or heteroarenes (including the new metabolites **1, 2** and **8** reported here) are formed with e.e. values over the range < 5 to >98% despite previous reports[2,4,5,7] of exclusive formation of homochiral *cis*-dihydrodiols of PAHs. (b) The benzylic R and non-benzylic S configuration previously reported for *cis*-dihydrodiols of PAHs is generally preferred in diols derived from heteroarenes (**1, 2, 7, 8, 9**) although exceptions (e.g. **10**) may occur.

Acknowledgement. We thank the SERC (Biotechnology Directorate) (NDS, JC), the DED/TBNI (NDS) and DENI (RB and RASMcM) for financial support.

References

1. Jerina, D.M., Daly, J.W., Jeffrey, A.M. and Gibson, D.T. Arch. Biochem. Biophys., 1971, 142, 394.
2. Jeffrey, A.M., Yeh, H.J.C., Jerina, D.M.,Patel, T.R., Davey, J.F and Gibson, D.T. Biochemistry, 1975, 14, 575.
3. Jerina, D.M., Selander, H., Yagi, H., Wells, M.C. Davey, J.F. Mahadevan, V. and Gibson, D.T. J. Am. Chem. Soc., 1976, 98, 5988.
4. Akhtar, M.N., Boyd, D.R., Thompson, N.J., Koreeda, M., Gibson, D.T., Mahadevan, V.and Jerina, D.M. J. Chem. Soc., Perkin Trans. 1, 1975, 2506.
5. Koreeda, M., Akhtar, M.N., Boyd, D.R., Neill, J.D., Gibson, D.T. and Jerina, D.M. J. Org. Chem., 1978, 43, 1023.
6. Gibson, D.T., Mahadevan, V., Jerina, D.M., Yagi, H. and Yeh, H.J.C. Science, 1975, 189, 295.
7. Jerina, D.M., van Bladeren, P.J., Yagi, H., Gibson, D.T., Mahadevan, V., Neese, A.S., Koreeda, M. Sharma, N.D. and Boyd, D.R. J. Org. Chem., 1984, 49, 3621.
8. Boyd, D.R., McMordie, R.A.S., Porter, H.P., Dalton, H., Jenkins, R.O. and Howarth, O.W. J. Chem. Soc. Chem. Commun., 1987, 1722.
9. Cerniglia, C.E., Morgan, J.C. and Gibson, D.T. Biochem. J., 1979, 180, 175.
10. Laborde, A.L. and Gibson, D.T. Appl. Environ. Microbiol., 1977, 34, 783.
11. Boyd, D.R., McMordie, R.A.S., Sharma, N.D., Dalton, H., Williams, P. and Jenkins, R.O. J. Chem. Soc. Chem. Commun., 1989, 339.
12. Boyd, D.R., Dorrity, M.R.J., Hand, M.V., Malone, J.F., Sharma, N.D., Dalton, H., Gray, D.J. and Sheldrake, G.N. J. Am. Chem. Soc., 1991, 113, 666.
13. Boyd, D.R., Sharma, N.D., Boyle, R. and McMordie, R.A.S. Unpublished data.

(Received in UK 18 December 1991)