Stereospecific Benzylic Hydroxylation of Bicyclic Alkenes by *Pseudomonas putida*: Isolation of (+)-*R*-1-Hydroxy-1,2-dihydronaphthalene, an Arene Hydrate of Naphthalene from Metabolism of 1,2-Dihydronaphthalene

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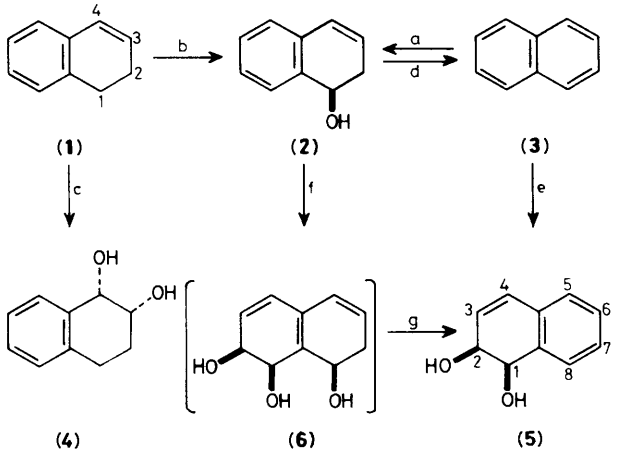
Metabolism of the bicyclic alkenes 1,2-dihydronaphthalene, indene, and 1,2-benzocyclohepta-1,3-diene by a mutant strain of *Pseudomonas putida* yields benzylic monols (exclusively with *R* configuration) as major metabolites and vicinal *cis* diols as minor products having an excess of the *S* configuration at the benzylic position.

Over the last century arene hydrates [*e.g.* compound (**2**)] have been implicated (although never identified) as transient metabolites of naphthalene (**3**), (step a) and 1,2-dihydronaphthalene (**1**) (step b) in mammals.[1] The formation of arene hydrate (**2**) as an initial metabolite of naphthalene (**3**) was inferred from both the liberation of naphthalene (**3**) only after acidification of the mammalian urine,[1] and the isolation of crystalline glucosiduronic acid conjugates of naphthalene hydrate (**2**) of varying diastereoisomeric purity from the urine of different animals.[1] Despite further evidence that arene hydrates of anthracene,[2] phenanthrene,[2] quinoline,[3] and benzo[*e*]pyrene[4] are formed as metabolites in mammalian systems, the assumed instability of these arene hydrates has, to date, precluded their isolation or determination of structure and absolute stereochemistry. This communication describes the first isolation and unequivocal structural assignment to an arene hydrate metabolite.†

Addition of 1,2-dihydronaphthalene (**1**) to a growing culture of a mutant strain (UV4) of *Pseudomonas putida* and product isolation after 22 h, yielded the expected *cis*-tetrahydrodiol (**4**) (step c). However, contrary to expectations, the latter product proved to be a relatively minor metabolite (10—15%) which was accompanied by the *cis*-dihydrodiol (**5**) as the major component (85—90%). The optical rotation values ($CHCl_3$) for the *cis*-diol metabolites (**5**) ($[\alpha]_D$ + 246°; 1*R*, 2*S*) and (**4**) ($[\alpha]_D$ + 39°; 1*S*, 2*R*) allied to both h.p.l.c. and n.m.r. analysis of the di-2-methoxy-2-phenyl-2-trifluoromethylacetate (diMTPA) derivatives confirm that both metabolites were of very high optical purity [≥98% enantiomeric excess (e.e.)] and of opposite absolute configurations.

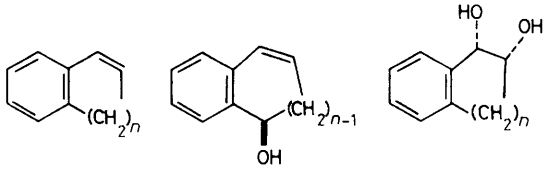


The unexpected formation of the *cis*-dihydrodiol (**5**) as a major metabolite could be explained by (i) enzyme-catalysed benzylic hydroxylation to yield an arene hydrate of naphthalene (**2**) (step b), (ii) spontaneous or enzymatic dehydration to yield naphthalene (**3**) (step d), (iii) enzymatic formation of *cis*-dihydrodiol (**5**) from naphthalene (**3**) as reported previously[5,6] (step e).

Since the initial metabolism of 1,2-dihydronaphthalene (**1**) in *P. putida* showed little evidence of the proposed intermediates (**2**) and (**3**), the study was repeated but products were isolated at an earlier stage (3 h). Rapid extraction (dichloromethane) followed by n.m.r. analysis of the crude product mixture (300 MHz, $CDCl_3$) showed the presence of the *cis*-diols (**5**) (*ca.* 15%) and (**4**) (*ca.* 20%) with the arene hydrate (**2**) as the major metabolite (*ca.* 60%). The naphthalene hydrate (**2**) which was isolated and purified by preparative t.l.c. was a crystalline solid (m.p. 52—53 °C) which was found to be chromatographically and spectrally (i.r., n.m.r., m.s.) indistinguishable from an authentic sample.[7,8] The optical rotations of the naphthalene hydrate (**2**) (Table 1) and product of hydrogenation in ethanol (H_2, Pd/C), 1-tetralol ($[\alpha]_D$ −31°, $CHCl_3$), combined with the n.m.r. and h.p.l.c. analysis of the MTPA ester derived from the 1-tetralol, all indicate that the arene hydrate (**2**) was of very high enantiomeric excess (≥98% ee) and of the (1*R*) configuration.

A pure sample of naphthalene (**3**), resulting from metabolism of 1,2-dihydronaphthalene (**1**) (< 5% total metabolites), was obtained and identified by g.l.c.–m.s. analysis after preparative t.l.c. purification and removal of the traces of alkene substrate (**1**) remaining after bromination (Br_2/CCl_4).

Further evidence that the *cis*-dihydrodiol metabolite (**5**) of 1,2-dihydronaphthalene (**1**) was obtained *via* steps b, d, and e



n = 1	(**7**)	(**8**)	(**9**)
n = 2	(**1**)	(**2**)	(**4**)
n = 3	(**10**)	(**11**)	(**12**)

† Recent experiments[8] on the metabolism of 1,4-dihydronaphthalene by *P. putida* have shown the formation of two further types of arene hydrates of naphthalene, 2-hydroxy-1,2-dihydronaphthalene, and 1-hydroxy-1,4-dihydronaphthalene.

Table 1

	Monol product				Diol product			
Substrate	% Yield[a]	$[\alpha]_D$[b]	% E.e.[c]	Configuration[d]	% Yield[a]	$[\alpha]_D$[b]	% E.e.[c]	Configuration[d]
(7)	(8) 12[e]	−178°	≥98	$1R^{9,12}$	(9) 47[e]	−11°	20	$1S,2R^{9}$
(1)	(2) 61[f]	+52°	≥98	$1R^{10}$	(4) 22[f]	+39°	≥98	$1S,2R^{5,11}$
(10)	(11) 90	+160°	≥98	$7R^{12,13}$	(12) 10	+25°	≥98	3S,4R

[a] Relative yield from n.m.r. analysis of crude extract. [b] $CHCl_3$ solvent. [c] Based upon $[\alpha]_D$ comparisons with literature values and h.p.l.c.–n.m.r. analysis of MTPA derivatives. [d] Note: The benzylic *R* configuration in compounds (**8**), (**2**) and (**11**) is stereochemically identical to the benzylic *S* configuration in compounds (**9**), (**4**) and (**12**), due to a change in substituent priorities during application of the sequence rule. [e] Accompanied by indan-1-one (41%) and several minor metabolites. [f] Accompanied by *cis*-dihydrodiol (**5**; 17%).

was sought using [4-^{2}H]1,2-dihydronaphthalene (**1**) as substrate. The isolated naphthalene (**3**) was found to contain one ^{2}H atom per molecule and the *cis*-dihydrodiol metabolite (**5**) was found to contain a proportion of the ^{2}H- label at positions 1,4,5, and 8 by ^{1}H n.m.r. spectroscopy (400 MHz, CD_3OD and $CDCl_3$) in support of the proposed metabolic sequence. A more accurate determination of the % ^{2}H in (**5**) found at positions 1,4,5, and 8 was obtained by ^{2}H n.m.r. spectroscopy. While the % ^{2}H found at positions 1, 4, and 8 were almost equivalent (17—24% ^{2}H), the value at position 5 was much larger (52% ^{2}H). The latter isotope pattern may be rationalised in terms of a concomitant metabolic sequence (in addition to steps b → d → e) involving the formation of a *cis*-dihydrodiol derivative (**6**) from the arene hydrate (**2**) followed by spontaneous or enzymatic dehydration to yield the *cis*-dihydrodiol (**5**) (steps b → f →g). The quest for further evidence of the putative triol metabolite (**6**) is currently in progress.

The ability of *P. putida* to yield an arene hydrate metabolite (**2**) from dihydroarene (**1**) is in accord with the similar benzylic hydroxylation reactions observed when the five (**7**; $n = 1$) and seven-membered (**10**; $n = 3$) bicyclic alkene analogues of 1,2-dihydronaphthalene (**1**; $n = 2$) are used as substrates (Table 1). Of the hydroxylated metabolites shown in Table 1, only the arene hydrate (**2**) and *cis*-diol (**12**) have not previously been obtained in optically active form. The absolute configuration of *cis*-diol (**12**) was assigned as (3*S*,4*R*) based upon a stereochemical correlation sequence to the alcohol metabolite (**11**).[8,13]

Previous observations have indicated that dioxygenases are able to catalyse mono-oxygenation reactions[6,14—16] by a mechanism which is at present obscure. Indeed, it was recently observed[15] that the F39/D strain of *P. putida* produced (1*S*)-indenol (**8**) from indene with a low optical purity (26% e.e.). In the present communication we report that the UV4 strain of *P. putida* catalysed benzylic mono-hydroxylations yielding the opposite (*R*) configuration exclusively (≥98% e.e.). Our observations that diol metabolites (**4**), (**9**) and (**12**) have a consistent benzylic *S* configuration, and are of variable optical yield, agree with those of Wackett *et al.*[15] who obtained compound (**9**) with a similar configuration and optical purity. It has previously been shown[15] that purified toluene dioxygenase was able to monohydroxylate both indan and indene (**7**) and thus was not due to adventitious mono-oxygenases which may be present in whole cell preparations. Since our observations represent the first report of the enzyme-catalysed production of an optically pure alcohol from an alkene, it will clearly be of interest to determine whether the highly stereoselective monohydroxylation is a consequence of the enzyme structure or substrate (or both). The mechanistic details underlying this phenomenon are under investigation in our laboratories.

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