Pergamon

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Biohydroxylation of 2-naphthoic acid by *Pseudomonas testosteroni*: absolute configuration of (1*R*,2*S*)-2-carboxy-*cis*-1,2-dihydro-1,2-dihydroxynaphthalene

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Abstract: The absolute stereochemistry of the 2-carboxy-*cis*-1,2-dihydro-1,2-dihydroxynaphthalene, a diol produced by *Pseudomonas testosteroni* A3C, was shown by X-ray crystal analysis to be 1*R*,2*S* © 1997 Elsevier Science Ltd. All rights reserved.

Biohydroxylations continue to be of interest to synthetic organic chemists[1,2]. With appropriate mutant strains of microorganisms single isomer products are easily accumulated in ≥95% yield and ≥98% *ee*. Since Gibson's initial discovery of *cis*-1,2-dihydrodiols by bacterial oxidations of arenes, e.g. toluene **1**, naphthalene **2**[5–7], several analogous dihydroxylations have been described for benzoic acids[8–12] and *o*-phthalic acids[13–15]. These compounds have been used for a wide variety of elegant syntheses, notably by Hudlicky[1], Ley[16], Johnson[4] and Carless[3].

While studying a complete catabolic pathway for 2-naphthoate by *Burkholderia* sp. it was established that 1-hydroxy-2-naphthoate was formed as the substrate for the putative dioxygenative cleavage of the substitued ring between carbons 1 and 2[17]. It seemed possible that a 2-carboxy-1,2-dihydro-1,2-dihydroxynaphthalene would be the initial oxidation product, and then dehydrated to 1-hydroxy-2-naphthoate.

Knackmuss *et al.*[18,19] described the formation of a *cis*-diol, 2-carboxy-1,2-dihydro-1,2-dihydroxynaphthalene **6**, by a strain of *Pseudomonas* sp. A3C, which grew with 2-naphthalene sulphonate, but which could hydroxylate 2-naphthoate as an isostere substrate, and the product accumulated quantitatively because this bacterium did not possess a diol dehydrogenase, necessary for the formation of 1,2-dihydroxynaphthalene, the product obtained directly by dihydroxylation of the sulphonate.

We prepared **6** to test this hypothesis and found that it was neither oxidized by whole cells of *Burkholderia* sp. JT1500 nor transformed by their cell extracts. However, we have determined the absolute configuration of **6** by X-ray crystal analysis as 1*R*,2*S*, confirming a *cis*-configuration expected. It is still possible that the antipode of **6** is an intermediary metabolite in the conversion of 2-naphthoate to 1-hydroxy-2-naphthoate by *Burkholderia* sp. JT1500, but we have no access to this. It seems unlikely that either of the two *trans*-diastereomers are intermediates, since *trans*-1,2-diols are not known to be formed from arenes by bacteria.

The absolute stereochemistry found for **6** is the same as those *cis*-diols derived from toluene **1**, naphthalene **2** and benzoic acid **3**, but antipodal to that from 4-bromobenzoic acid **4** with respect to the alkyl or halo-substituent. However, the tertiary centre is the same as that for D-quinic acid **5** and of **3**, and thus provides a bicyclic chiral intermediate for synthetic applications (Scheme 1).

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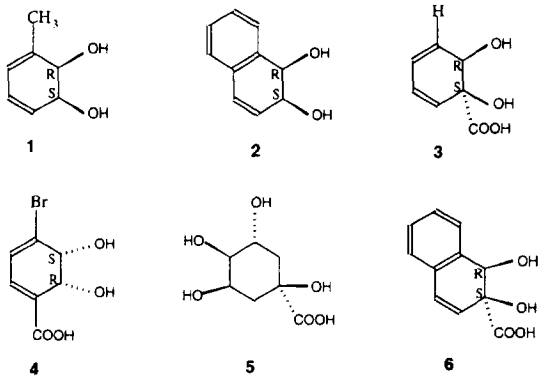
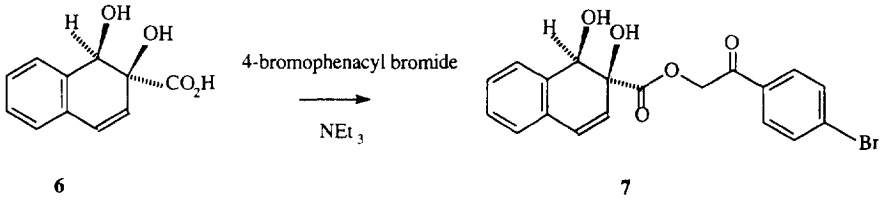


Scheme 1. Absolute structures of different dihydrodiols.

The title diol **6** is also not a substrate for the benzoate-1,2-dihydrodiol dehydrogenase obtained from other strains of bacteria e.g. *P. putida* PaW1, even though so many analogues of the benzoic acid diols **3**, including 3,4-disubstituted substrate diols, are readily oxidized with decarboxylation to catechols.

Diol **6** was derivatized with 4-bromophenacyl bromide to give **7** for the X-ray analysis (Scheme 2). The crystalline compound was subjected to X-ray diffraction analysis (Figure 1). The *ee* of the diol was estimated by NMR chiral shift procedures with Mosher's reagents on the derived 4-bromophenacyl ester[20]. Examination suggested an enantiomeric excess of ≥98% similiar to that of other arene-*cis*-diols produced by bacterial oxidations



Scheme 2. Derivatization of 2-naphthoate-1,2-dihydrodiol.

Experimental

Preparation of 2-naphthoate-1,2-dihydrodiol 6

Pseudomonas testosteroni A3C was grown in mineral salts media containing salicylate (10mM) as carbon source and inducer, 2-naphthoate (1mM) as substrate and citrate (0.1%). The diol **6** accumulation was monitored by UV and HPLC. After 150 hours the 10 l culture was harvested, the supernatant concentrated to 1 l by evaporation under reduced pressure, acidified to pH 2.2 and

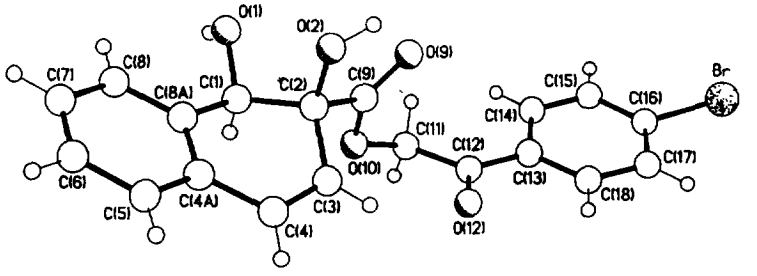


Figure 1. X-ray structure of **7**.

extracted with ethylacetate and crystals obtained [^{1}H-NMR data (d_4-methanol) in ppm: 5.2 (1H, d, J=2Hz); 5.95 (1H, dd, J=9, 2Hz); 6.68 (1H, dd, J=9, 2Hz); 7.1–7.35 (4H, m); 7.6 (1H, m), UV data: λ_{max}=260nm, m.p.=147°C,$[\alpha]_D^{20}$ = 24,0±0,3 (MeOH)]. The diol was isolated with a yield of 81%. **6** Decomposed on heating under acidic conditions (pH 1) to give 1-hydroxy-2-naphthoic acid.

Derivatization of 2-naphthoate-1,2-dihydrodiol ***6***

A solution of the diol (1 mmol) in dry acetone (4 ml) was neutralised by portion-wise addition of redistilled triethylamine. A solution of 4-bromophenacyl bromide (1,2 eq.) in acetone (5 ml) was added and the solution allowed to stand overnight at room temperature. The solution was diluted with water, stirred and the resultant precipitate collected by filtration. The crude solid was purified by silica gel chromatography and recrystallised from CH_2Cl_2/PE to give colourless needles [^{1}H-NMR ($CDCl_3$)in ppm: 5.4 (1H, s); 5.55 (2H, d, J=2Hz); 6.15 (1H, d, J=8Hz); 6.75 (1H, d, J=8Hz); 7.1–7.4 (4H, m); 7.7 (2H, d, J=6Hz); 7.8 (2H, d, J=6Hz); ^{13}C-NMR ($CDCl_3$) in ppm: 191.7, C_q; 174.8, C_q; 135.2, C_q; 133.5, 132.6, 131.8, 130.2, 129.5, 129.2, 127.9, 127.6, 127.2, 125.4, 124.5, CH; 131.2, C_q; 75.6, C_q; 72.8, CH; 67.4, CH_2; m.p.=149°C].

Notes

Crystal data for 7

$C_{19}H_{15}BrO_5$, M=403.2, monoclinic, space group *P*2, *a*=10.009 (1), *b*=5.123 (3), *c*=16.927 (1) Å, β=98.18 (1)°, *V*=859.2 (5) Å^3, *Z*=2, D_c=1.56 gcm^{-3}, μ (M_o–K_α)=24.2 cm^{-2}, *F* (000)=408. A clear needle of dimensions 0.67×0.32×0.04 mm was used. 1652 Independent reflections were measured with a Siemens P4/PC diffractometer with M_o–K_α radiation (graphite monochromator) using ω-scans. The structure was solved by direct methods and all the non-hydrogen atoms were refined anisotropically using full matrix least squares based on F^2 with absorption corrected data to give R_1=0.069, wR_2=0.172 for 1074 independently observed reflections [$|F_o|>4\sigma$ ($|F_o|$), $2\theta \leq 50°$] and 234 parameters. The absolute stereochemistry (1*R*,2*S*) was determined unambigously by an *R*-factor test [R_1^+=0.069, R_1^-=0.073]. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB12 1EW.

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