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RESOLUTION AND ASSIGNMENT OF ABSOLUTE CONFIGURATION TO THE (+)- AND (-)-CIS AND TRANS 3,4-DIOL METABOLITES OF THE ANTI-JUVENILE HORMONE PRECOCENE I

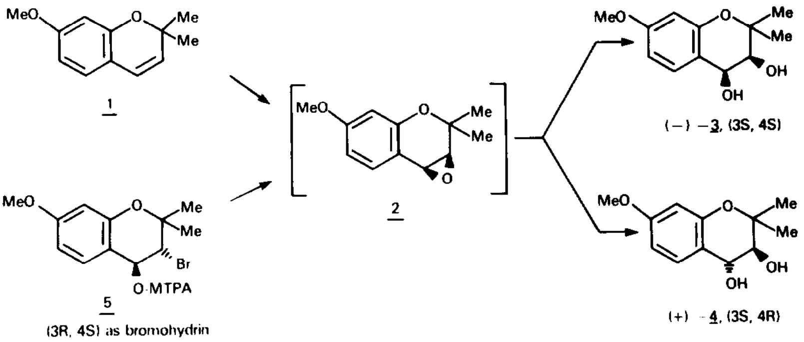
Rita A. Halpin, Shaaban F. El-Naggar, Kenneth M. McCombe†, Kamlesh P. Vyas,
Derek R. Boyd†*, and Donald M. Jerina*

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases,
National Institutes of Health, Bethesda, MD 20205 U.S.A.
and
†Department of Chemistry, The Queen's University of Belfast, Belfast BT9 5AG, N. Ireland

Summary: The cis and trans 3,4-diol metabolites of precocene I have been prepared in optically pure form and their absolute configurations have been assigned by independent nmr and CD techniques.

The plant isolate precocene I (7-methoxy-2,2-dimethyl-2H-benzo[b]pyran, 1, Scheme)[1a] shows marked growth regulatory effects on a range of insects[1b] and causes necrosis of their corpora allata. The necrosis has been linked to covalent binding of metabolically formed precocene 3,4-oxide (2) to cellular macromolecules.[1c] This remarkably reactive epoxide (2) also undergoes spontaneous hydrolysis to cis (3) and trans (4) 3,4-diols in water.[2] Parallel has been drawn[1c,2] between the bioactivation of 1 in insects and the formation of carcinogenic bay-region diol epoxides from polycyclic aromatic hydrocarbons in mammals.[3] In order to determine the stereochemical course of the metabolism of 1 in mammals, the present report describes the resolution and assignment of absolute configuration to the precocene diols 3 and 4 (Scheme).

SCHEME



The precocene diols[2] were obtained by treating precocene I (1 g in 10 ml CH_2Cl_2) with m-chloroperoxybenzoic acid (3 g in 50 ml CH_2Cl_2) for 1 hr at rt. The resulting m-chlorobenzoate adducts of 2 were hydrolyzed (3 g NaOH in 40 ml of 50% methanol-water for 0.5 hr at rt) to the diols which were separated by HPLC on a Du Pont Zorbax SIL column (21.2 x 250 mm) eluted with 20% THF in CH_2Cl_2 (k' 0.60 and 1.14) and crystallized from ether. The less polar isomer (3, 133 mg, mp 98-99°) was assigned cis stereochemistry and the more polar isomer (4, 129 mg, mp 120-121°) trans stereochemistry based on their NMR spectra (220 MHz, $CDCl_3$); H_3 3.59 and H_4 4.79 δ with $J_{3,4}$ = 4.1 Hz for cis 3, H_3 3.54 and H_4 4.48 δ with $J_{3,4}$ = 8.0 Hz for trans 4. The analytically pure diols had ε_{280} = 3015 in ethanol. Resolution of 3 and 4 proved far more difficult than had been anticipated. Attempts to form bis-esters of the diols with the acid chlorides of either (-)-menthoxyacetic acid (MAA) or (-)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) in pyridine led to complex mixtures. A major breakthrough was had, however, in the finding that the bis-MAA esters were formed from the diols in acceptable yield when dry triethylamine was used as the solvent. In a typical experiment, 200 mg of 4, 1.1 g of MAA-Cl, and 1.7 ml of triethylamine were maintained at 50° for 24 hr. The mixture was diluted with an equivalent volume of methanol and chromatographed on a Du Pont Zorbax ODS column (21.2 x 250 mm) eluted with methanol to isolate the diastereomer mixture (k' = 3.2, 354 mg). Separation of the diastereomers was achieved with a pair of Zorbax SIL columns (21.2 x 250 mm) eluted with 10% ether in cyclohexane:

<u>diastereomers of trans diol (4)</u>

less polar (k' 0.91), $[\alpha]_D$ -116° (THF, c = 35 mg/ml); (+)-4, $[\alpha]_D$ +44° (ethanol, c = 33 mg/ml)
more polar (k' 1.07), $[\alpha]_D$ -55° (THF, c = 43 mg/ml); (-)-4, $[\alpha]_D$ -45° (ethanol, c = 29 mg/ml)

<u>diastereomers of cis diol (3)</u>

less polar (k' 1.28), $[\alpha]_D$ -97° (THF, c = 80 mg/ml); (+)-3, $[\alpha]_D$ +7.5° (ethanol, c = 8 mg/ml)
more polar (k' 1.44), $[\alpha]_D$ -21° (THF, c = 71 mg/ml); (-)-3, $[\alpha]_D$ -7.2° (ethanol, c = 18 mg/ml)

Dispite the difficulty of these separations (α = 1.12 - 1.17), the high efficiency of these HPLC columns permitted good recovery (>70%) of the individual compounds. All four isomers (>99% purity) were obtained as colorless, viscous oils which were hydrolyzed to the requisite cis (mp 82-84°) and trans (colorless oil) diols by treatment with NaOH (10% by wt.) in 30% methanol-THF for 15 min at rt. Relationship of the diastereomers to their corresponding diols is indicated above.

NMR spectra (220 MHz, C_6D_6) of the diastereomeric bis-esters of trans 4 provided a preliminary indication of their absolute configuration. For the less polar diastereomer with the more negative $[\alpha]_D$, the diastereotopic $-CH_2$-hydrogens of the pair of $-CO-CH_2-O-$ in the (-)-menthoxyacetyl groups appear as a singlet at 4.13 δ and an AB-quartet centered at 3.97 and 4.06 δ with $J_{gem} \sim 16$ Hz. For the more polar diastereomer, each of the $-CH_2-$ signals appears as an AB-quartet in which H_A and H_B are centered at 3.87 and 4.02 δ and at 3.99 and 4.13 δ with J_{gem} = 16 Hz in each case. For both diastereomers, $J_{3,4}$= 6.0 Hz. By analogy with several related bis-MAA esters of polycyclic aromatic hydrocarbon trans diols,[4] (+)-trans 4 derived from the less polar diastereomer would be predicted to have (3S,4R)-absolute configuration.[5] Further support for the assignment of (3S,4R)-absolute configuration to (+)-4 was obtained from the CD spectrum of its cuprammonium complex (Cupra A),[6] a technique previously used for cyclic trans diols[7] in which the dihedral angle between the hydroxyl groups does not exceed 60°. Observation of the requisite weak negative Cotton effect in the 560-600 nm wavelength region indicates a λ configuration which requires (3S,4R)-absolute configuration for (+)-4 (Figure).

A completely independent method of resolution and assignment further confirmed the above results for 4 and additionally allowed assignment of the enantiomers of 3. In this approach (Scheme, absolute configuration as shown), <u>trans</u>-3-bromo-4-hydroxyprecocene I[2] was prepared by treating 1 (3.6 g) with N-bromoacetamide (3.1 g in 60 ml of 30% water in THF) for 7 hr at rt in the dark under N_2; 5.0 g, mp 68-70° from hexane. The bromohydrin (3.0 g in 10 ml of dry pyridine) was allowed to react with (-)-MTPA-Cl (3.0 g) for 17 hr at rt to provide an oily dia-

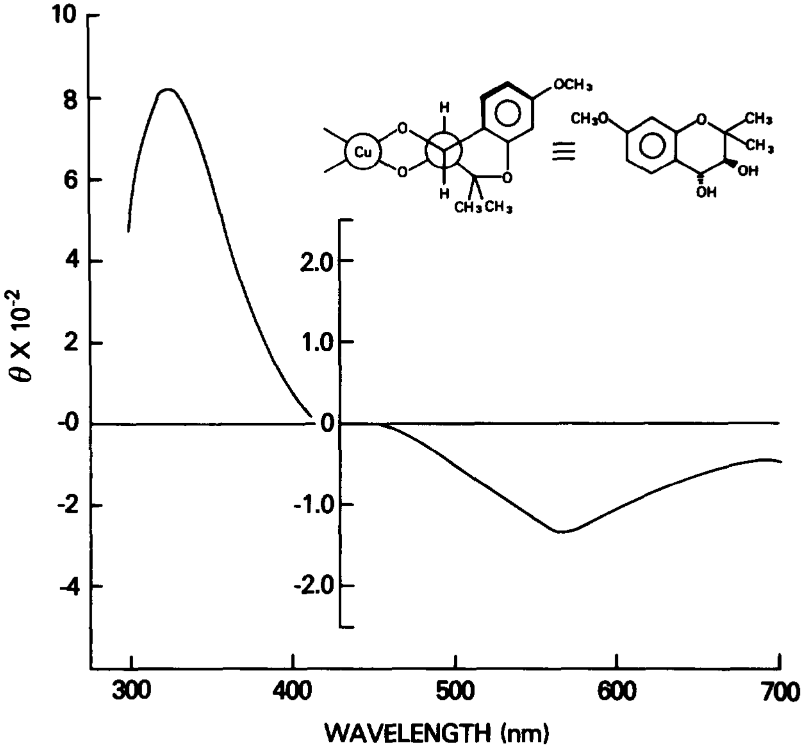


Figure. CD spectrum (Jasco J-500A spectropolarimeter) of 6mM (+)-(3S,4R)-trans-3,4-dihydroxyprecocene I and Cupra A in ethanol-water (1.39 mg (+)-4 in 0.38 ml of ethanol and a molar equivalent of copper ion in 0.62 ml of water).[6]

stereomeric mixture (3.5 g) of bromo esters from which a single isomer (5) crystallized from pentane; mp 99-101°, $[\alpha]_D$ +41° (c = 11.0 mg/ml, $CHCl_3$). Although the other diastereomer could not be obtained in pure form due to decomposition on silica gel, the enantiomer of 5 was readily isolated in pure form ($[\alpha]_D$ -39°) when the acid chloride of (+)-MTPA was used. Comparison of the NMR spectrum of 5 with that of its less polar diastereomer present in the mother liquor from the crystallization suggested that the bromohydrin precursor to 5 had (3R,4S)-absolute configuration when compared to related bromohydrin MTPA esters of several polycyclic aromatic hydrocarbons of known configuration;[8] H_3 4.27 δ with $J_{3,4}$ = 7.2 Hz for the more polar crystalline diastereomer and H_3 4.20 δ with $J_{3,4}$ = 6.1 Hz for the less polar diastereomer in the mother liquor. Treatment of 5 with 2% NaOH in 50% THF-water for 14 hrs at rt provided (+)-trans-4 and (-)-cis-3. Since reaction of 5 under basic conditions is presumed to proceed via 2 which suffers cis and trans hydrolysis[2,9] at C-4, (+)-(3S,4R)-4 is the expected trans diol and (-)-cis-3 must have (3S,4S)-absolute configuration.[10]

In summary, the present report describes the resolution of the cis and trans 3,4-diols (3 and 4) of precocene I. A combination of three spectral techniques all indicate that (-)-3 and (+)-4 have (3S,4S) and (3S,4R) absolute configuration, respectively. Preliminary studies of the metabolism of 1 with rat liver microsomes from 3-methylcholanthrene-treated rats indicate that 3 and 4 are major metabolites and that a 55% enantiomeric excess of (-)-4 is formed, presumably *via* the (+)-2 epoxide. Related cis and trans diols have been detected as mammalian metabolites of precocene II although their configurations have not been described.[11] Further studies on the mammalian metabolism of precocene I are in progress.

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5. It should be noted that because of the oxygen in the precocene I ring system, a change in the priority ranking of substituents at the chiral, nonbenzylic carbinol carbon occurs relative to related diols of polycyclic aromatic hydrocarbons. Thus, a trans-(R,R)-diol of a polycyclic aromatic hydrocarbon corresponds to the trans-(3S,4R)-diol of precocene I.

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