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SYNTHESIS, RESOLUTION AND ABSOLUTE CONFIGURATION OF THE DIOL METABOLITES OF PRECOCENE I

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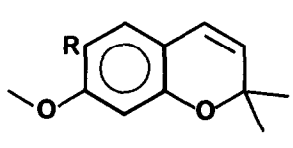
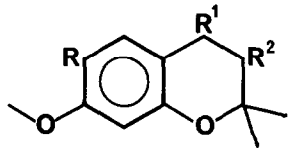
Summary: All four stereoisomers of precocene I 3,4-dihydrodiol have been prepared and their absolute configurations assigned by the exciton chirality method.

The chromene derivatives, precocene I (1) and II(2), which occur naturally in some *Ageratum* species, induce premature metamorphosis or sterility when administered to certain sensitive species of insect[1]. The compounds cause these effects by terminating the production of the juvenile hormones (JH) by selectively destroying the insects' *corpora allata*, the glands where these hormones are biosynthesised[2]. The highly reactive precocene epoxides (3)[3,4] appear to be the chemical species responsible for these biological effects and are thought to be formed in these glands by oxidation of the precocenes by the cytochrome P-450 linked JH epoxidase[5]. Studies[6] of the metabolism of [4-^{3}H]precocene I by *in vitro* preparations of *corpora allata* from *Locusta migratoria* have shown that the *cis* and *trans* diols (4) are the sole low molecular weight metabolites produced and are formed stereospecifically[7]. Extensive labelling of macromolecular cell components occurs also and is attributable to the reaction of these epoxides with nucleophilic cell constituents. Necrosis of the glands results with consequent effects upon the insects' metamorphosis. In this paper the synthesis, resolution and the assignment of the absolute configuration of the diols is described.





(1) R = H
(2) R = OMe

(3) R = H or OMe, R^1R^2 = O
(4) R = H, R^1 = R^2 = OH
(5) R = H, R^1 = *m*$ClC_6H_4CO_2$, R^2 = OH
(6) R = H, R^1 = R^2 = OMTP
(7) R = H, R^1 = R^2 = OMA
(8) R = H, R^1 = R^2 = *p*$(CH_3)_2NC_6H_4CO_2$

The mixture of *cis* and *trans* *m*-chlorobenzoate half esters of the diols (5) was prepared as described previously[3] and was separated by preparative high performance liquid chromatography (hplc) into two components (Waters LC500, 2 silica prepPAK cartridges, 30% ether-pentane, 200 ml/min.). The first component, a viscous oil, was hydrolysed (1N NaOH, 50% ethanol-water, ambient temperature, 15 min) to a diol (m.p. 124.5 - 125.5°C, hexane-ethyl acetate) which was assigned the *trans* configuration on the basis of its n.m.r. spectrum after exchange with D_2O (100 MHz, $CDCl_3$, $J_{3,4}$ = 8Hz). The second component, a crystalline solid, (m.p. 105 - 106°C, pentane-ether) gave the *cis* diol (m.p. 101- 102°C, hexane-ethyl acetate, $J_{3,4}$ = 5Hz) under comparable conditions. The assignment of *cis* stereochemistry to the latter compound was confirmed as it had the same physical properties as the diol prepared by treating precocene I with osmium tetroxide (pyridine, ambient temperature, 3hr, sodium metabisulphite work-up).

An attempt was made to separate the enantiomers of the *cis* and *trans* diols as their diastereoisomeric *bis*(-)-α-methoxy-α-trifluoromethylphenylacetyl (MTP) derivatives (6). The esters were prepared by reacting the diols with the acid chloride[8] (pyridine, ambient temperature, 24hr) but although the *trans* derivatives were separable by hplc[9] (15% ether-hexane) the *cis* derivatives could not be separated under these conditions. The *bis*(-)menthoxyacetyl (MA) derivatives (7) were prepared by reacting the diols with the appropriate acid chloride[10] (pyridine, 45°, 6hr) and both pairs of diastereoisomers were found to be readily separated by analytical hplc (7% ether-hexane, 100% water saturated) as shown in the Table. Preparative scale separations were carried out (LC500, 1 prepPAK silica cartridge, 7% ether-hexane, 100% water saturated, 150 ml/min) and each of the four diastereoisomers was obtained as a viscous oil of greater than 95% purity as determined by analytical hplc.

TABLE: Rotations and Other Selected Physical Properties of the Dihydrodiols and Derivatives

	4, R_1,R_2 = OH	7, R_1,R_2 = (-)MAO	8, R_1,R_2 = DMAB
trans	+50.9 (C = 0.23, $CHCl_3$) oil	-107 (C = 0.3, $CHCl_3$) k' = 1.5	-298 (C = 0.08, MeOH)
	-50.8 (C = 0.09, $CHCl_3$) oil	- 35.5 (C = 0.3, $CHCl_3$) k' = 1.9	+289 (C = 0.06, MeOH)
cis	+33.6 (C = 0.15, $CHCl_3$) m.p. 81-83°C	-139 (C = 0.35, $CHCl_3$) k' = 3.35	+ 66.3 (C = 0.08, MeOH)
	-29.2 (C = 0.15, $CHCl_3$) m.p. 77.5-81.5°C	- 25.5 (C = 0.41, $CHCl_3$) k' = 4	- 51.5 (C = 0.08, MeOH)

Reduction of each of the menthoxyacetyl derivatives with lithium aluminium hydride (tetrahydrofuran, ambient temperature, 1hr) gave the corresponding optically active diol (see Table) each of which was purified by preparative centrifugal chromatography[11] (0.1% methanol-ether) and then converted to their *bis*-*p*-N,N-dimethylaminobenzoates (8) by reaction with the acid chloride[12] (pyridine, catalytic amount of 4-N,N-dimethylaminopyridine, 100°C, 24hr). These derivatives were purified using the same method as for the diols (1% methanol-dichloromethane) and recycling was necessary to separate the *cis* derivatives from the *p*-N,N-dimethyl-

aminobenzoic anhydride which was invariably present in the crude reaction product. Their chemical purity was checked by hplc (20% hexane-dichloromethane, 100% water saturated) and their circular dichroism (C.D.) spectra measured (Figure).

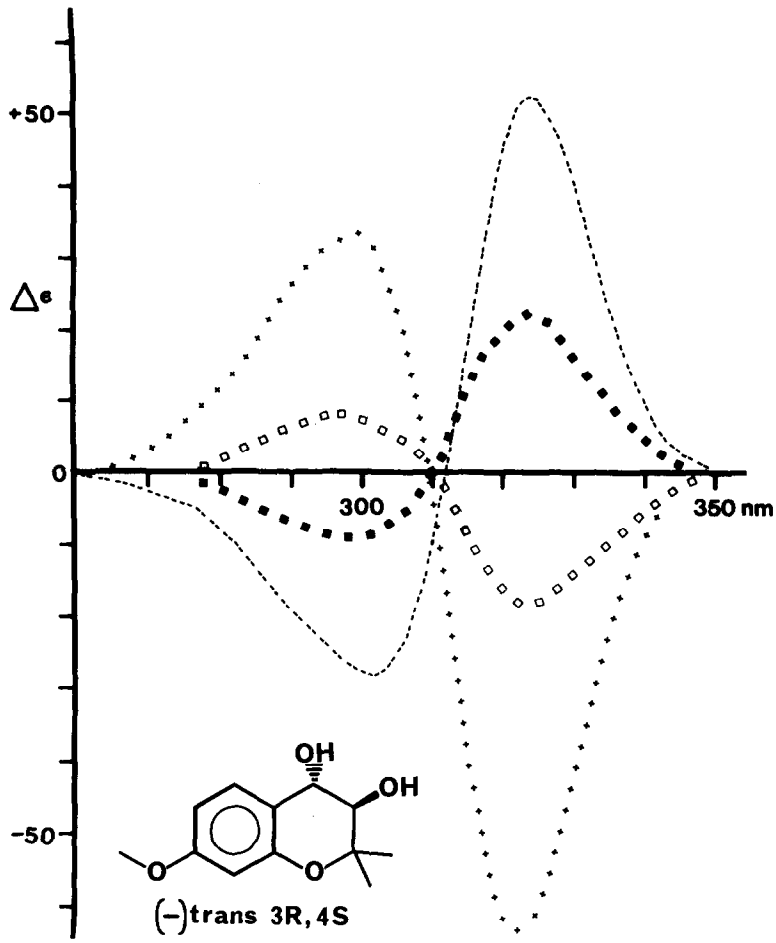


FIGURE: C.D. spectra of the (+)*cis* (■ ■ ■ ■), (-)*cis* (□ □ □ □), (+)*trans* (+ + + +) and (-)*trans* (--------) *bis-p*-N,N-dimethylaminobenzoate derivatives of 3,4-dihydrodiols of precocene I in methanol (2 mg/25 ml).

Each pair of enantiomeric derivatives provided mirror-image curves and the absolute configuration of each enantiomer can be assigned from the sign of the peak at the longer wavelength[13]. A positive sign indicates a clockwise spatial relationship between the interacting benzoate chromophores and vice versa. Thus, the derivative of the (-)-*trans* diol has a positive peak and may be assigned the 3*R*,4*S* configuration (see Figure). In a similar manner the (+)-*trans* diol may be assigned the 3*S*,4*R* configuration and the (-)-*cis* and (+)-*cis* the 3*S*,4*S* and 3*R*4*R* configurations respectively. The identical assignments of the configurations

of the (+)-*trans* and (-)-*cis* diols have been made independently[14] using different techniques in connection with studies on the mammalian metabolism of precocene I. The *cis* and *trans* dihydrodiols have been shown to be metabolic products of precocene II produced by both insects[15] and by rat liver microsomes[16] but their absolute configurations have not been determined.

The application of the methods described here to the diol metabolites of precocene I produced by insects will be described elsewhere.

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