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The Absolute Configuration of the Four Stereoisomers of *trans*-Anethole Diol (1-(4′-Methoxyphenyl)-1,2-propanediol), a Metabolite of Anethole in the Rat

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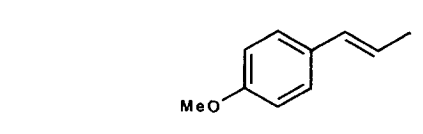
Abstract: The four stereoisomers of anethole diol, one of the major neutral metabolites of *trans*-anethole in the rat, have been separated by HPLC of their camphanyl esters and characterized by ^{1}H-NMR and CD spectroscopy. Their configurations were determined by comparison of their CD spectra with those of the 1-phenylpropane-1,2-diols. Copyright © 1996 Elsevier Science Ltd

trans-Anethole (1-(4′-methoxyphenyl)-prop-1-ene) **1**, a pleasant aromatic substance, is present in the oils of a large number of herbs and spices, notably anise. It is consumed in a wide range of foods, ranging from the vegetable fennel, anise and dill flavoured dishes common in Chinese cuisine to aniseed candies and the anise alcoholic beverages (Pastis) beloved in Mediterranean countries[1]. While its use as an added flavour in candies etc. results in a human dose of 65 μg/day, anise beverages contain 40 mg anethole as well as 7.2 g of ethanol[2]. A number of studies on the genotoxicity of *trans*-anethole have been published over last 15 years[3] and *trans*-anethole failed to induce DNA repair either in *Bacillus subtilis* or in isolated rat hepatocytes. The marked cytotoxicity of *trans*-anethole to rat hepatocytes is due to its epoxide metabolite[4], and a major detoxication pathway of *trans*-anethole epoxide **2** is hydration by cytosolic epoxide hydrolase giving anethole diol **3** with two stereogenic centres[4]. Although there have been several papers on the synthesis of anethole epoxides (*threo*- and *erythro*-)[5] and racemic anethole diol derivatives[6,7], there have been no reports of the isolation, characterization and physical properties of the four stereoisomers of *trans*-anethole diol. We now present the isolation of the four isomers of anethole diol in the form of their camphanyl esters. The absolute configurations of the [(1*S*,2*R*)-, or (1*R*,2*S*)-]-*erythro*- and [(1*R*,2*R*)- or (1*S*,2*S*)]-*threo*-diols **3** have been assigned on the basis of ^{1}H-NMR and CD spectral data. The determination of the configurations of the anethole diols provides the means for the study of the metabolic formation of each stereoisomer from *trans*-anethole in mammals.



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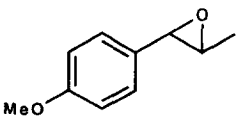

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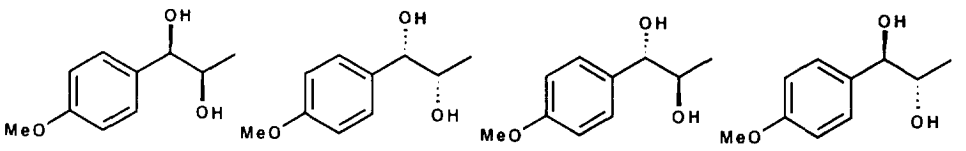


(1*R*, 2*R*)-3 (1*S*, 2*S*)-3 (1*S*, 2*R*)-3 (1*R*, 2*S*)-3

Experimental

Anethole diol was provided by Glidco Organics, Jacksonville, Florida, U.S.A. Silica gel 60 (0-200 μ) (Fisons) for column chromatography, precoated Kieselgel 60 F_{254} TLC plates (20 cm x 20 cm, cat.no. 5554, Merck), and magnesium sulfate (97 %) (Aldrich) were used. Solvents were analytical grade and dried over molecular sieves before use. HPLC used a Philips 4003 pump system, a Waters Associates M440 UV detector equipped with a 280 nm filter and a Shimadzu Chromatopac C-R6A as a recorder. The systems were: 1) SpheriSorb ODS-2 Excel column (25cm x 4.6 mm i.d., 5 μ particle size), mobile phase water-methanol (6 : 4 v/v), flow rate 1 ml/min for the separation of the *erythro-* and *threo-* isomers of anethole diol; 2) Daicel Chiralcel OJ column (25 cm x 4.6 mm i.d., 10 μ particle size), mobile phase *n*-hexane-2-propanol (9:1, v/v), flow rate; 0.5 ml/min, to separate the *threo-* [(*R*, *R*)- or (*S*,*S*)-] isomers of anethole diol; 3) Lichrosorb Si60 column (10 cm x 4 mm i.d., 5 μ particle size), mobile phase *n*-hexane-ethyl acetate (4:1 v/v), flow rate 1 ml/min, for the four dicamphanyl esters of anethole diol. ^{1}H-NMR Spectra were obtained with a JEOL 500MHz instrument, CD spectra with a JEOL J-600 instrument, and optical rotations with a Perkin-Elmer 141 polarimeter. FAB-MS spectra were obtained using a VG Analytical ZAB-2SE FAB mass spectrometer fitted with a cesium ion gun operated at 20-25 kV and using monothioglycerol as the matrix. Data acquision and processing were performed with VG Analytical Opus-AE software.

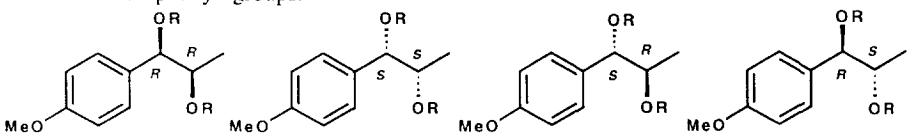
The Separation of the Four Stereoisomers of trans-*Anethole Diol* **3**

Separation of *threo-* and *erythro-* isomers of anethole diol **3** was achieved by HPLC in System 1, the two peaks having R_t 8.7 and 12.7 mn. These two compounds both had $[\alpha]_D$ +0.2 ($CHCl_3$), showing them to be the *erythro-* and *threo-* isomer pairs. The *threo-* pair [(*R*,*R*)-**3** and (*S*,*S*)-**3**] were separated by chiral HPLC in System 2 with *R*t 40.1 min, and had $[\alpha]_D$ -34.67 (*c*, 1.3, $CHCl_3$) and $[\alpha]_D$ +48.85 (*c*, 1.5, $CHCl_3$) , respectively. Their CD spectra are presented in Fig.1. The *erythro-* pair [(*S*,*R*)-**3** and (*R*,*S*)-**3**] could not be resolved on this column, even with changes in mobile phase composition.

Anethole diol (100 mg) was mixed with (-)-(*S*)-camphanyl chloride (150 mg) in anhydrous pyridine (5 ml) and left for 24 h at room temperature[8]. The mixture was poured into water (10 ml) and extracted with chloroform (3 x 2 ml) which was washed successively with satd. $NaHCO_3$ (2 ml), 0.1 M HCl (2 ml), satd. $CuSO_4$ (4 x 2ml) and water. The $CHCl_3$ was dried over $MgSO_4$ and evaporated under reduced pressure to give anethole diol

dicamphanyl esters **4** (150 mg). These were separated by TLC with *n*-hexane:benzene: ethyl acetate (9:5:7 v/v/v) and four spots were detected on UV light under I_2 vapour, R_f 0.44 (dicamphanyl esters), 0.31 and 0.19 (both monocaphanyl esters), and 0.08 (unreacted diols). These spots were scraped from the plates, eluted with $CHCl_3$ and examined by ^{1}H-NMR.

The ^{1}H-NMR of the spot with R_f 0.44 showed it to be a mixture and HPLC in System 3 gave four clearly separated peaks P-1, P-2, P-3, and P-4 (Rt 26.7, 32.5, 35.8, and 45.3 min) due to the four dicamphanyl esters , (1*S*,2*R*)-4, (1*R*,2*S*)-4, (1*R*,2*R*)-4 and (1*S*,2*S*)-4 respectively. All the camphanyl esters decomposed on GC-MS so their FAB-MS spectra were recorded. The four dicamphanyl esters of anethole diol all gave the same mass spectral patterns with the following ions: m/z 345 [fragment ion + H]$^+$, 346.1[isotope ion of fragment ion + H]$^+$, 543[M (= $C_{30}H_{38}O_9$) + H]$^+$, 560.3[M + NH_4]$^+$, 565[M + Na]$^+$, and 1102.5[2M + NH_4]$^+$ (Fig.1). The IR spectrum of each camphanyl ester showed two strong ester absorptions at 1780-90 and 1730-1752 cm^{-1}, attributable to two camphanyl groups.



R = (*S*)-camphanyl

(1*R*, 2*R*)-4 (1*S*, 2*S*)-4 (1*S*, 2*R*)-4 (1*R*, 2*S*)-4

Table 1. ^{1}H-NMR and CD spectral characteristics of the dicamphanyl esters of the four stereoisomers of *trans*-anethole diol

HPLC peak	Dicamphanyl ester	Rotation $[\alpha]_D$	CD curve	J_{AB} J_{BC} Hz	Assignment
P-1	(1S,2R)-4	-21.08 c, 1.66	-	4.03 6.23	*S*,*R*
P-2	(1R,2S)-4	+28.27 c, 1.42	+	4.76 6.60	*R*,*S*
P-3	(1R,2R)-4	+27.19 c, 4.13	+	7.70 6.60	*R*,*R*
P-4	(1S,2S)-4	-47.44 c, 5.19	-	7.69 6.23	*S*,*S*

^{1}H-NMR: P-1: (1*S*, 2*R*); 7.364 and 6.901 (each 2H, d, J=8.79 Hz), 6.086 (1H, d, J=4.03 Hz; -C_6H_4-C$\underline{H}^1$(O-camph)-C^{2}H(O-camph)), 5.385 (1H, q, -C^{1}H(O-camph)-C$^2\underline{H}$(O-camph)-C$^3H_3$), 3.806 (3H, s, -OCH_3), 2.398, 2.001, 1.907, 1.673 (each 2H, m; camphanyl group), 1.121, 1.093, 1.070, 1.010, 0.995, 0.863 (each 3H, s; camphanyl group), 1.258 (3H, d, J=6.23 Hz, -CH_3).

P-2: (1*R*, 2*S*); 7.318 (2H, d, J=8.43 Hz; aromatic), 6.882 (2H, d, J=8.79 Hz; aromatic), 6.014 (1H, d, J=4.76 Hz; -C_6H_4-C$\underline{H}^1$(O-camph)-C^{2}H(O-camph)), 5.407 (1H, q, -C_6H_4-C^{1}H(O-camph)-C$^2\underline{H}$(O-camph)-C$^3H_3$), 3.802 (3H, s, -OCH_3), 2.454, 2.331, 2.059, each 1H, m; camphanyl group), 1.923, 1.681 (each 2H, m;

camphanyl group), 1.103, 1.093, 1.024, 1.007 (each 3H, s; camphanyl group), 0.857(6H, s; camphanyl group), 1.299 (3H, d, J=6.60 Hz, $-CH_3$).

P-3: (1*R*, 2*R*); 7.316 (2H, d, J=8.43 Hz; aromatic), 6.887 (2H, d, J=8.79 Hz; aromatic), 5.869 (1H, d, J=4.76 Hz; $-C_6H_4-C\underline{H}^1$(O-camph)-C^2H(O-camph)), 5.418 (1H, q, $-C_6H_4-C^1H$(O-camph)-$C^2\underline{H}$(O-camph)-C^3H_3), 3.803 (3H, s, $-OCH_3$), 2.426, 1.986 (each 2H, m; camphanyl group), 1.903, 1.670 (each 2H, m; camphanyl group), 1.160, 1.081, 1.028, 0.987, 0.956, 0.830 (each 3H, s; camphanyl group), 1.163 (3H, d, J=6.60 Hz, $-CH_3$).

P-4: (1*S*, 2*S*); 7.316 (2H, d, J=8.79 Hz; aromatic), 6.902 (2H, d, J=8.79 Hz; aromatic), 5.892 (1H, d, J=4.76 Hz; $-C_6H_4-C\underline{H}^1$(O-camph)-C^2H(O-camph)), 5.480 (1H, q,$-C_6H_4-C^1H$(O-camph)-$C^2\underline{H}$(O-camph)-C^3H_3), 3.814 (3H, s, $-OCH_3$), 2.398 (2H, m; camphanyl group), 1.950 (4H, m; camphanyl group), 1.670 (2H, m; camphanyl group), 1.110, 1.081, 1.076, 0.977, 0.941, 0.815 (each 3H, s; camphanyl group), 1.166 (3H, d, J=6.23 Hz, $-CH_3$).

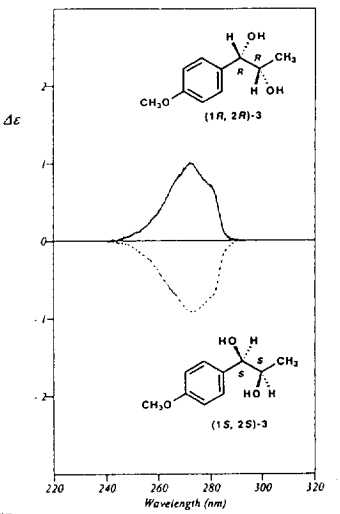


Figure 1

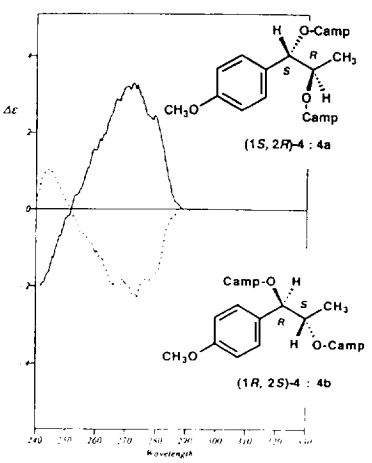


Figure 2a

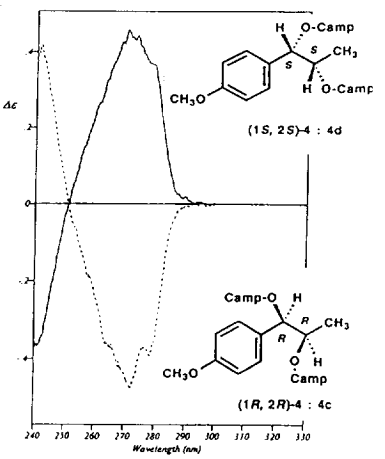


Figure 2b

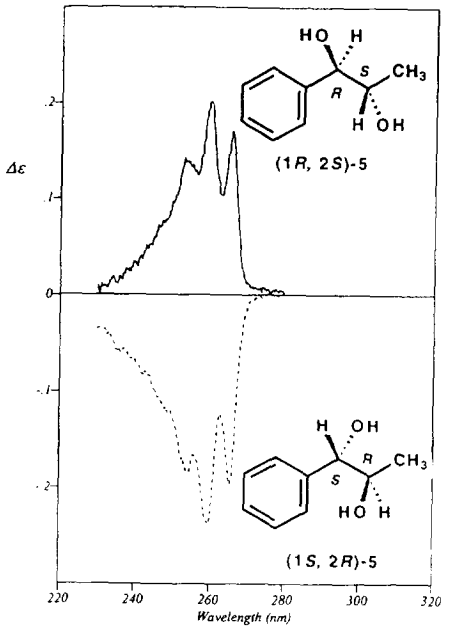


Figure 3a

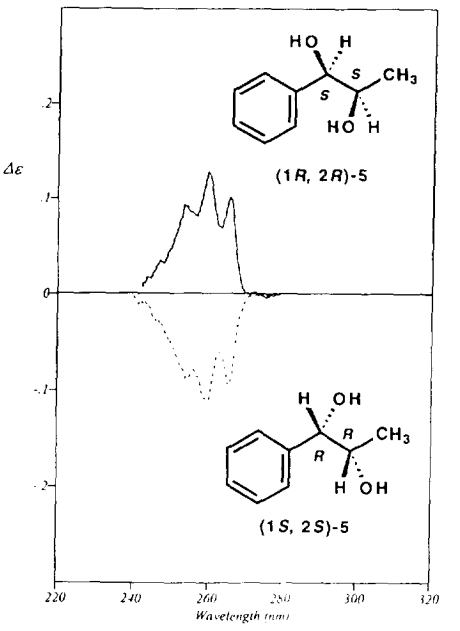


Figure 3b

These ^{1}H-NMR spectra allowed the assignment of the relative stereochemistries of the C^1- and C^2-carbon atoms from the coupling constants J_{AB} between the proton A (H_A) adjacent to the phenyl group and the proton B (H_B) adjacent to the terminal methyl group (Table 1). These values suggest that P-1 and P-2 are *erythro*- (J_{AB} = 4.03 and 4.76 Hz) and P-3 and P-4 are *threo*-forms (J_{AB} = 7.70 and 7.69 Hz). The J_{AB} values for the *erythro*-forms of 1-phenylpropane-1,2-diol are 3.9 Hz (*R*, *S*) and 4.3 (*S*, *R*) Hz and 7.3 Hz for both *threo*-forms (*R*,*R* and*S*,*S*)[9,10]. CD spectroscopy allowed the absolute stereochemistry of the α–carbon atom to be assigned. The CD spectra of the four dicamphanyl esters are shown in Figure 2a (*threo*) and 2b (*erythro*). Each shows aromatic ^1L_b bandS around 270 nm. The combination of these results with the coupling constant J_{AB} of the root proton of C_1 enabled the configuration of C_1 and C_2 atoms to be assigned (Table 1). For comparison, the CD curves of authentic samples of the four stereoisomers of 1-phenylpropane-1,2-diol **5** [(1*S*, 2*R*)-, (1*R*, 2*S*)-, (1*R*, 2*R*)- and (1*S*, 2*S*)-**5**], whose configurations are known[9,10], are presented in Figs. 3a (*erythro*) and 3b (*threo*). These correspond well with those of the anethole diols **3** and their camphanyl esters **4**. As a consequence, the absolute configurations of the six compounds are [(1*R*, 2*R*), (1*S*, 2*S*)-**3** and (1*S*, 2*R*), (1*R*, 2*S*), (1*R*, 2*R*), (1*S*, 2*S*)-**4**] were fully assigned on the basis of combined NMR and CD analyses.

DISCUSSION

A comparison of the NMR and CD spectra of the four stereoisomers of the dicamphanates of 1-(4-methoxy)-phenylpropane-1,2-diol **4** with those of 1-phenylpropane-1,2-diol **5** established the absolute stereochemistry of four anethole diols and their dicamphanyl esters. Gillard and Michell[11] reported many examples of the CD spectra of α–hydroxyphenyl derivatives and demonstrated that the negative sign associated with the CD of the longest wavelength aromatic transition (^1L_b) can be correlated with the *S*-configuration at the

α–carbon atom (positive sign correlates with *R*). The methoxy group of the 4′-methoxyphenyl analogues being *para* does not affect the CD interpretation although it does lead to an expected loss in fine structure. CD below 250nm observed for the dicamphanyl esters is associated with the ester and lactone chromophores of the camphanyl group. The reported coupling constants for 1-phenyl-1,2-propanediol [*erythro* J_{AB} = 3.9Hz (*R*, *S*), 4.3 Hz (*S*, *R*), and *threo* J_{AB} = 7.3 Hz (*R*, *R*), 7.3 Hz (*S*, *S*) in $CDCl_3$] agreed well with the present findings reported here for the 4′-methoxyphenyl analogues. The combination of HPLC separation of the dicamphanyl esters of chiral diols with CD and NMR spectroscopy thus provides a very effective method for the characterization of stereoisomeric diol metabolites from alkene epoxides.

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