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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ISOMERIC DIHYDRODIOLS OF POLYCYCLIC HYDROCARBONS

THE EFFECT OF CONFORMATION ON ELUTION ORDER

B. TIERNEY*, P. BURDEN, A. HEWER, O. RIBEIRO and C. WALSH

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London SW3 6JB (Great Britain)

H. RATTLE

Portsmouth Polytechnic, White Swan Road, Portsmouth (Great Britain)

and

P. L. GROVER** and P. SIMS

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London SW3 6JB (Great Britain)

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SUMMARY

The orders in which K-region and non-K-region dihydrodiols derived from benz[*a*]anthracene, 7-methylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, dibenz[*a*,*h*]anthracene and benzo[*a*]pyrene elute from a Partisil 5 column in cyclohexane–ethanol (98:2) have been examined using high-performance liquid chromatography. The elution profiles obtained show that, within a series of dihydrodiols derived from any one hydrocarbon, those *trans*-dihydrodiols that are known to exist predominantly in a preferred quasi-diequatorial conformation elute much earlier, presumably because of intramolecular hydrogen bonding, than do those that for steric reasons, are known to exist in a predominantly quasi-diaxial conformation. These differences appear to be sufficiently large to permit the conformation of an uncharacterized dihydrodiol to be predicted from its relative retention time on high-performance liquid chromatography.

INTRODUCTION

Interest in the preparation and properties of dihydrodiol derivatives of polycyclic hydrocarbons increased following reports that certain non-K-region dihydro-

* Present address: Department of Biochemistry, University of Vermont, Burlington, Vt. 05401, U.S.A.

** To whom correspondence should be addressed.

diols appear to be involved in the metabolic activation of carcinogenic polycyclic hydrocarbons through their conversion into reactive vicinal diol-epoxides[1–4]. The preparation of mixtures of isomeric dihydrodiols derived from several different polycyclic hydrocarbons by the use of an ascorbic acid–ferrous sulphate–EDTA system has been described[5–7]. The dihydrodiols were separated and purified using high-performance liquid chromatography (HPLC) on a column of Partisil 5 eluted with cyclohexane–ethanol prior to characterization and nuclear magnetic resonance (NMR) spectrometry and it became apparent that the order in which the different dihydrodiols derived from a single hydrocarbon eluted from a column of Partisil 5 was dependent upon their conformation. This paper describes the results obtained in experiments in which the chromatographic characteristics of dihydrodiols derived from six polycyclic hydrocarbons were examined on HPLC under our chromatographic conditions and were then considered in relation to their known conformations.

EXPERIMENTAL

Materials

Samples of the *trans*-1,2-, 3,4-, 5,6-, 8,9- and 10,11-dihydrodiols derived from benz[*a*]anthracene[7], the *trans*-1,2-, 3,4-, 5,6-, 8,9- and 10,11-dihydrodiols derived from 7-methylbenz[*a*]anthracene[5], the *trans*-3,4-, 5,6-, 8,9- and 10,11-dihydrodiols derived from 7,12-dimethylbenz[*a*]anthracene[7], the *trans*-4,5-, 7,8-, 9,10- and 11,12-dihydrodiols and the *cis*-2a,3-diol derived from 3-methylcholanthrene[6], the *trans*-1,2-, 3,4- and 5,6-dihydrodiols derived from dibenz[*a,h*]anthracene[8] and the *trans*-4,5-, 7,8-, 9,10- and 11,12-dihydrodiols derived from benzo[*a*]pyrene[9] were obtained from the parent hydrocarbons using an ascorbic acid–ferrous sulphate–EDTA oxidation system as previously described and were characterized by examination of their UV, mass and ^{1}H-NMR spectral properties.

High-performance liquid chromatography

Mixtures of dihydrodiols derived from each polycyclic hydrocarbon were separated using a DuPont 830 instrument fitted with a Model 837 variable wavelength UV detector (DuPont, Hitchin, Great Britain) and a Partisil 5 silica column (250 mm × 5 mm I.D.) (Whatman, Maidstone, Great Britain), which was eluted with HPLC grade cyclohexane (Rathburn Chemicals, Walkerburn, Great Britain)–ethanol (dried and redistilled before use) (98:2). Samples (0.5 ml) were applied as solutions in cyclohexane–tetrahydrofuran (3:1), the eluting solvent was passed at a flow-rate of 1.6 ml/min at 40° and the eluent monitored for UV absorption at an appropriate wavelength.

RESULTS AND DISCUSSION

trans-Dihydrodiols derived from polycyclic aromatic hydrocarbons are known to adopt a preferred quasi-diequatorial conformation in the absence of other factors[10–13] and dihydrodiols adopting this conformation elute earlier on HPLC under our chromatographic conditions, presumably because of intra-molecular hydrogen bonding which reduces their polarity, than do dihydrodiols adopting a predominantly quasi-diaxial conformation. The results obtained, when the behaviour of dihydrodiols

related to benz[*a*]anthracene was examined, are shown in Fig. 1. The four dihydrodiols known to exist in a predominantly diequatorial conformation[11,13], the *trans*-3,4-, 5,6-, 8,9- and 10,11-diols, eluted earlier than the related *trans*-1,2-diol, which, as a "bay-region" diol, is hindered and exists in a quasi-diaxial conformation[13]. When

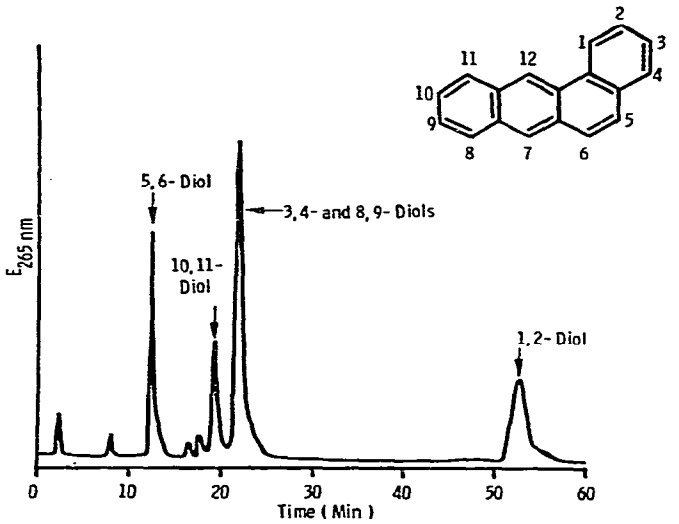

Fig. 1. The elution profile obtained when a mixture of the *trans*-dihydrodiols derived from benz[*a*]anthracene were subjected to HPLC using a Partisil 5 silica column (250 mm × 5 mm I.D.) eluted with cyclohexane–ethanol (98:2). The sample (0.5 ml) was applied as a solution in cyclohexane–tetrahydrofuran (3:1) the eluting solvent was passed at a flow-rate of 1.6 ml/min at 40° and the eluate monitored for UV absorption.

the HPLC elution characteristics, under our chromatographic conditions, of dihydrodiols derived from 7-methylbenz[*a*]anthracene were examined (Fig. 2), the main change that was found in the elution order, compared with that for the diols derived from the unsubstituted hydrocarbon, concerned the delayed elution of the 5,6- and 8,9-dihydrodiols. This was not unexpected since these two diols are known to exist predominantly in a quasi-diaxial conformation[5,14], presumably because of steric interactions between the hydroxyl groups on either the 6- or the 8-positions and the adjacent methyl substituent at the 7-position.

Results in agreement with those obtained with 7-methylbenz[*a*]anthracene and with NMR data[7] were obtained when the elution order of the *trans*-dihydrodiols derived from 7,12-dimethylbenz[*a*]anthracene was examined (Fig. 3). Here the 10,11-diol also eluted in a position consistent with its existence in a predominantly quasi-diaxial conformation[7], presumably because of interaction with the adjacent 12-methyl substituent. The 1,2-dihydrodiol derived from 7,12-dimethylbenz[*a*]anthracene has not been detected either as a metabolite[7,15] or as a major product of oxidation of the hydrocarbon[7], possibly because of strong steric hindrance, and therefore has not been examined, but it would be expected to exist in a diaxial conformation. The presence of a methyl group in the 12-position is known to distort the benz[*a*]anthracene molecule so that the 1,2,3,4 ring no longer lies in the same plane as the anthracene

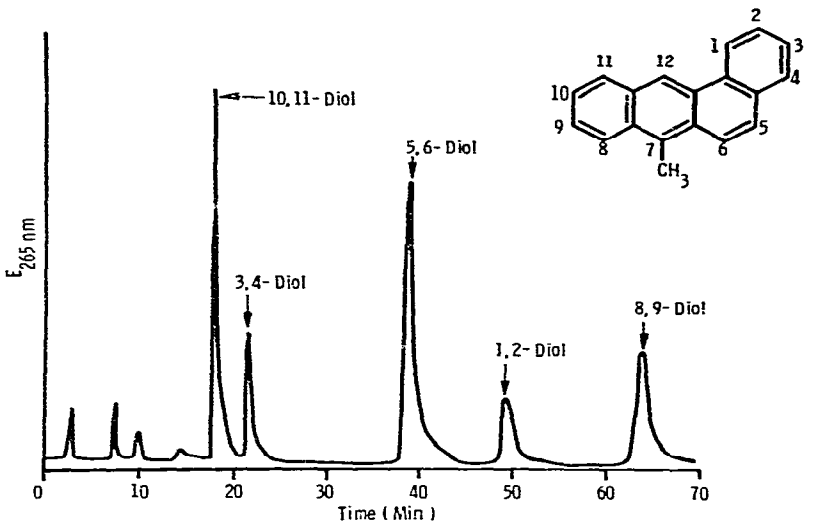

Fig. 2. The elution profile obtained when a mixture of the *trans*-dihydrodiols derived from 7-methylbenz[*a*]anthracene was subjected to HPLC exactly as described in the text and in the legend to Fig. 1.

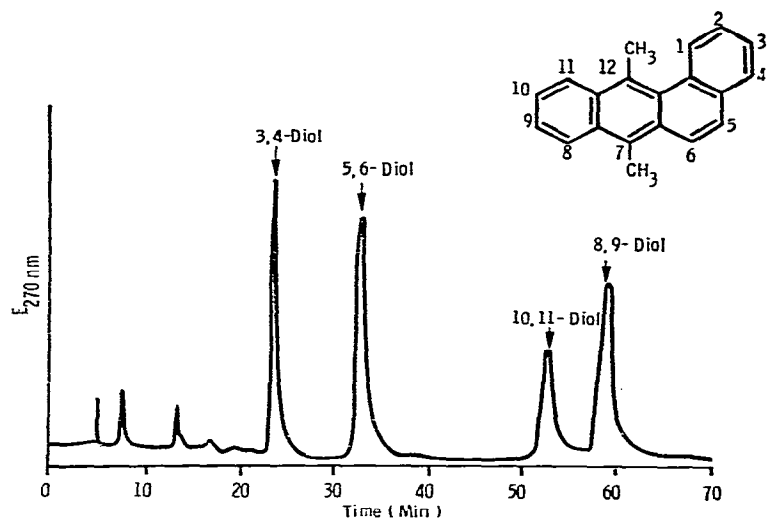

Fig. 3. The elution profile obtained when four *trans*-dihydrodiols derived from 7,12-dimethylbenz[*a*]-anthracene were subjected to HPLC exactly as described in the text and in the legend to Fig. 1.

nucleus[16,17]. Fig. 4 shows the HPLC elution profile obtained with the dihydrodiols derived from 3-methylcholanthrene, which is also in agreement with NMR data[6,18]. The *trans*-4,5- and 9,10-dihydrodiols exist in the preferred quasi-diequatorial conformation and elute early, whilst the "bay-region" 7,8-diol elutes much later because of its quasi-diaxial conformation. The *trans*-11,12-dihydrodiol also elutes early in a position that is consistent with its quasi-diequatorial conformation and it is evident from comparisons between both the NMR data on, and the chromatographic

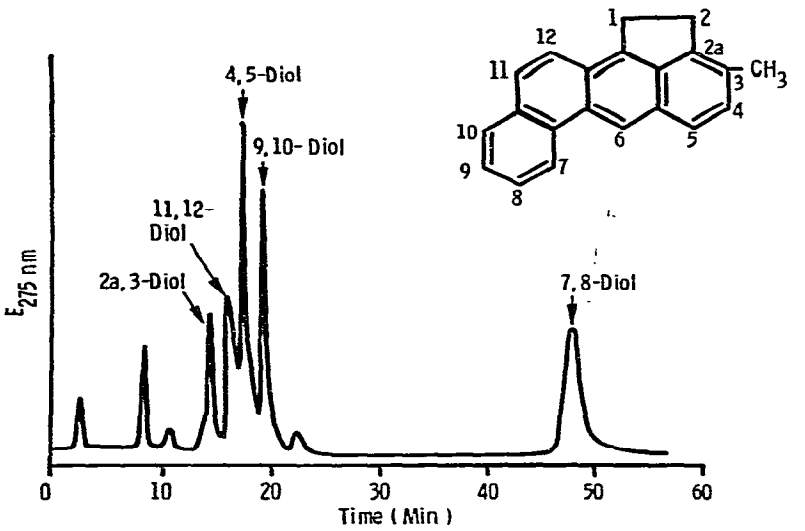

Fig. 4. The elution profile obtained when a mixture of dihydrodiols derived from 3-methylcholanthrene was subjected to HPLC exactly as described in the text and in the legend to Fig. 1.

behaviour of, the K-region dihydrodiols of 3-methylcholanthrene and 7-methylbenz-[*a*]anthracene that, whilst methyl substitution at the 7-position of 7-methylbenz[*a*]-anthracene results in the adoption of a predominantly quasi-diaxial conformation, the presence of the 1,2-methylene bridge in 3-methylcholanthrene has a much smaller effect on the conformation of the corresponding K-region 11,12-diol. The conformation of the *cis*-2a,3-diol has not been investigated.

The relationships between HPLC elution order, under our chromatographic conditions, and conformation for the dihydrodiols derived from the other two hydrocarbons studied, dibenz[*a*,*h*]anthracene (Fig. 5) and benzo[*a*]pyrene (Fig. 6), are simpler since these hydrocarbons are unsubstituted. The "bay-region" 1,2-dihydrodiol of dibenz[*a*,*h*]anthracene exists predominantly in the quasi-diaxial conformation[8] and elutes considerably later than the other two quasi-diequatorial diols, the 3,4- and 5,6-derivatives. Within the series of dihydrodiols related to benzo[*a*]pyrene, the quasi-diequatorial K-region 4,5-diol[14] elutes first and is followed by the other diol, the 7,8-diol, that can adopt this preferred conformation[10,11]. The second K-region diol, the 11,12-derivative elutes later than these two diequatorial diols and presumably exists in a predominantly quasi-diaxial conformation because of its position adjacent to a "bay-region" in the molecule; there do not appear to be any NMR data available for the 11,12-diol that might support this conclusion. The other "bay-region" diol derived from benzo[*a*]pyrene, the 9,10-diol, exists in a predominantly quasi-diaxial conformation[10,11] and elutes considerably later than the other *trans*-diols (Fig. 6).

The data that have been obtained for the different *trans*-dihydrodiols derived from six polycyclic hydrocarbons that are presented here and that relate their elution order to their conformation appear to be consistent. Within any series of related *trans*-dihydrodiols, it appears that K-region diols in the preferred quasi-diequatorial conformation are the least polar and elute first under our chromatographic conditions and that these are followed by any non-K-region diols that are also predominantly

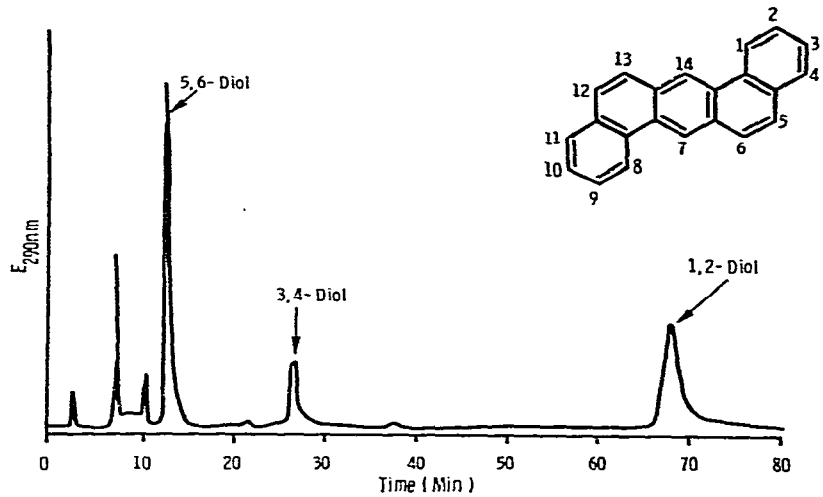

Fig. 5. The separation of three *trans*-dihydrodiols of dibenz[*a*, *h*]anthracene using HPLC as described in the text and in the legend to Fig. 1.

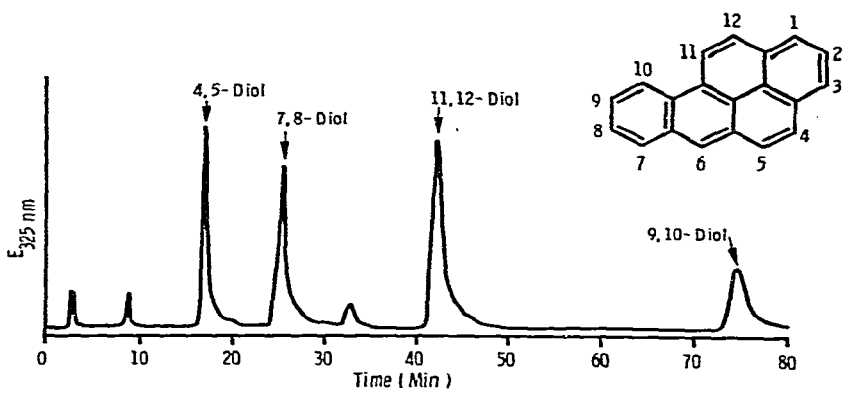

Fig. 6. The elution profile obtained when a mixture of four *trans*-dihydrodiols derived from benzo[*a*]pyrene were subjected to HPLC as described in the text and in the legend to Fig. 1.

quasi-diequatorial. K-Region diols existing, because of steric interactions, in a predominantly quasi-diaxial conformation elute next and are followed by the more polar non-K-region diols that are in a predominantly quasi-diaxial conformation. Those non-K-region diols that have one hydroxyl group adjacent to a methyl substituent are the most polar of all and it appears that the presence of an adjacent methyl group can have a greater effect on diol conformation than an adjacent "bay-region", a point that is illustrated by the elution characteristics of the 8,9- and 1,2-dihydrodiols of 7-methylbenz[*a*]anthracene (Fig. 2). The results reported here are in good general agreement with others that have been obtained for dihydrodiols derived from unsubstituted hydrocarbons[19]. Those differences that do exist between the two sets of data may well be due to the distinctly different column adsorbents and eluting solvents that were used. The reason why K-region diols in a particular conformation

invariably appear to elute before any non-K-region diols that are also in that conformation is not clear at present, however. The generalization mentioned above may be useful in permitting predictions to be made about the identity of uncharacterized dihydrodiol metabolites of a polycyclic hydrocarbon from comparative HPLC elution data in so far as they may be tentatively classified as K- or non-K-region dihydrodiols existing in predominantly quasi-diequatorial or diaxial conformations. The factors that affect the order in which dihydrodiols elute on HPLC under any particular chromatographic conditions are not sufficiently well understood at present to permit predictions to be made about the order in which related diols of a similar type that exist in a similar conformation will elute.

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