Optical Resolution of Mutagenic and Carcinogenic Derivatives of Polyaromatic Hydrocarbons by High Pressure Liquid Chromatography on a Chiral Support

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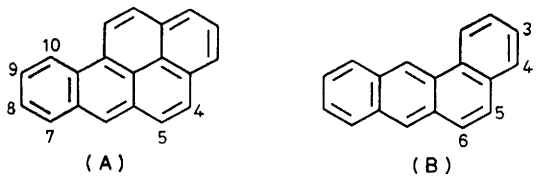
Summary Optically active diols of benzo[*a*]pyrene and benz[*a*]anthracene, and a benzo[*a*]pyrene diol–epoxide, were resolved by h.p.l.c. on (*R*)-(−)-2-(2,4,5,7-tetranitro-9-fluorylideneamino-oxy)propionic acid (TAPA) bonded to silica gel.

COVALENT binding of activated metabolites of the highly carcinogenic polyaromatic hydrocarbons (PAH) to DNA is widely considered to be the initiating step in their mutagenic and carcinogenic action, and is hence of considerable topical interest. Compounds to which attention has been drawn in this context are diols, epoxides, and diol–epoxides of PAH's, such as benzo[*a*]pyrene (BP), benz[*a*]anthracene (BA), and 7,12-dimethylbenz[*a*]anthracene. The formation of these derivatives generates chiral centres in the molecule, and it has been shown recently that the mutagenic and carcinogenic activity is enantioselective.[1,2] It has thus become of considerable importance to develop methods for the resolution of these oxygen-containing PAH derivatives.

Diastereomeric derivatives have been used successfully for the h.p.l.c. separation of these optical isomers.[3,4] For instance, the *trans*-7,8-dihydroxybenzo[*a*]pyrene (**1**) was resolved *via* the di-(+)-α-methoxy-α-trifluoromethylphenylacetate.[3] We now report a procedure which permits efficient separation of the enantiomers of a number of oxidized PAH's without the need for an esterification step.

Chiral 2-(2,4,5,7-tetranitro-9-fluorylideneamino-oxy)propionic acid (TAPA) is a well known resolving agent for optically active aromatic compounds capable of interacting with the former through charge transfer complexation.[5] Some years ago it was shown that this reagent can also be used in h.p.l.c. with the TAPA coated on, or covalently linked to, silica gel.[6,7] For the present work the support was prepared as follows: (*R*)-(−)-TAPA was first coupled to 3-aminopropyltriethoxysilane with *NN'*-dicyclohexylcarbodi-imide; the silane formed was then bonded to Lichrosorb Si 100 (5μ), and finally the remaining unchanged silanol groups were 'end-capped' with trimethylsilyl chloride. The slurry packing method was employed for column preparation. MeOH–CH_2Cl_2 was used as the mobile phase. Solvent composition and other chromatographic conditions employed are given in the Table.

TAPA was shown to manifest stereoselectivity for all compounds studied. Hydrocarbons (**1**)—(**6**) differ from each other by one or more structural features, *e.g.*, the molecular skeleton and position of the nonaromatic ring, the functionality, or the position or geometry of the vicinal diols. Correspondingly, the α-values vary quite considerably (Table).



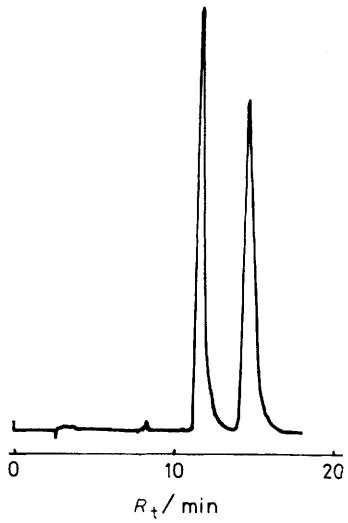


FIGURE 1. Resolution of (±)-*trans*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene (**2**); see the Table for experimental conditions.

TABLE. Resolution[a] of diols and diol–epoxides of polyaromatic hydrocarbons by h.p.l.c. on silica gel linked to (*R*)-(−)-TAPA.

PAH[b]	Mobile phase (MeOH : CH_2Cl_2)	k'_1	k'_2	α[d]
(**1**) *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene[c]	4 : 11	3·43	3·86	1·125
(**2**) *trans*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene	4 : 11	3·54	4·67	1·319
(**3**) *cis*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene	5 : 95	4·12	4·46	1·083
(**4**) *r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene	1 : 4	3·65	4·06	1·112
(**5**) *cis*-5,6-dihydroxy-5,6-dihydrobenz[*a*]anthracene[c]	5 : 95	1·98	2·14	1·081
(**6**) *trans*-3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene	5 : 95	5·85	6·22	1·063

[a] Chromatographic conditions: column dimensions = 15 × 0·46 cm; flow rate = 0·7 ml/min; ambient temperature. [b] For numbering, see the general formulae (A) and (B). [c] Both the racemic and (−)-enantiomeric compounds were available. [d] α = resolution factor = k_2/k_1 = ratio of capacity factors of the two enantiomers.

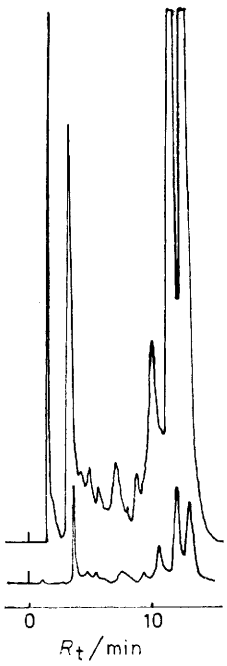


FIGURE 2. Resolution of (±)-*r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydro-benz[*a*]pyrene (**4**). The large peaks at the right correspond to the enantiomers of the main component; see the Table for experimental conditions.

The highest resolution factors were found for the BP *trans*-diols (**1**) and (**2**) [see Figure 1 for the excellent separation of (**2**)]. The *anti*-7,8-diol-9,10-epoxide of BP (**4**) is of special interest, because of its high carcinogenicity. Good resolution was obtained also in this case, as shown in Figure 2. The compound is known to be unstable and the additional peaks appearing in the chromatogram may be due to decomposition products.†

Change of the geometry of the diol groups from *trans*, in (**2**), to *cis*, in (**3**), reduces the α-value very considerably (Table). Also, the tetracyclic BA derivatives (**5**) and (**6**) have relatively low separation factors.

In the case of (**1**) and (**5**) the pure (−) isomers were available, and it was found that (−)-(**1**) and (+)-(**5**) were, respectively, the first enantiomers to emerge. The establishing of the relationships between structure and order of emergence[6,8] could be useful for the determination of the configuration of related compounds in these series.

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† The epoxide is normally not opened by methanol in the absence of acid. Nevertheless, the stability of (**4**) under the above conditions should be checked, although any decomposition will not change the enantiomeric ratio.

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