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Resolution of polycyclic aromatic hydrocarbon dihydrodiols via diastereomeric formaldehyde acetals

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Abstract: Diastereomeric formaldehyde acetals, formed from the reaction of racemic benzo[*a*]pyrene dihydrodiol and (−)-chloromethylmenthyl ether, are novel intermediates for effecting the convenient resolution of these metabolites by HPLC. This resolution technique seems generally applicable since the dihydrodiols of benzo[*c*]phenanthrene can also be readily resolved through this methodology. Key differences in the proton NMR spectra of the diastereomeric dihydrodiol menthyloxymethyl ethers have been identified which could be used for absolute stereochemical assignments. © 1997 Elsevier Science Ltd

Benzo[*a*]pyrene (BaP) is a carcinogenic hydrocarbon belonging to the environmentally ubiquitous group of polynuclear hydrocarbons. For experiments aimed at understanding BaP induced neoplasia, availability of its optically pure trans dihydrodiol and diol epoxide metabolites is critical. Originally, BaP dihydrodiol (±)-**1** has been resolved via the 7,8-*bis*-*O*-menthyloxyacetate[1] (MAA) and the 7-*O*-[(−)-α-methoxy-α-trifluoromethylphenylacetyl] (MTPA) ester.[2] Subsequently, lability of the intermediates as well as improved separation prompted the use of tetrahydrodiol *bis*-*O*-MAA derivatives.[3] However, in this approach to prepare the dihydrodiol enantiomers several manipulations (3 to 5 steps) are required on each resolved diastereomer. Enantioselective routes[4–6] to dihydrodiols are also labor intensive when both enantiomers are required. For us, an ideal route is one wherein facile separation of the enantiomers is achieved late in the synthesis. Although direct resolution of dihydrodiols by chiral HPLC has been achieved,[7] the inherent problems associated with semi-preparative chiral HPLC, such as leaching of the chiral phase and difficulties in achieving good resolutions, precluded us from pursuing this approach. Due to the foregoing considerations, we have developed a new and convenient resolution method based on the formation of diastereomeric formaldehyde acetals. This method has the potential for wide general applicability, particularly in instances where existing procedures are less than optimal.

Recently, both enantiomers of chloromethylmenthyl ether (CMME) have become commercially available, with the (−)-isomer being cheaper. Although (−)-CMME has been used in the measurement of enantiomeric excesses, to our knowledge, it has not been applied for preparative enantiomeric resolution.[8] The use of this isomer as a resolving agent seemed attractive for several reasons.

(1) A menthyloxymethyl ether (MOME) is simply a mixed formaldehyde acetal, which can be cleaved under a variety of mild conditions that are non-detrimental to sensitive molecules such as dihydrodiols.
(2) Compared to the MAA derivatives of hydrocarbon diols, which have a 4 atom separation between the nearest chiral centers in the hydrocarbon and menthyl moieties, the MOME derivatives have a 3 atom separation. Proximity of chiral centers is critical to good diastereomeric resolution.
(3) Separations using non-polar solvents and greatly improved peak shapes are an added advantage with the non-polar MOME derivatives.

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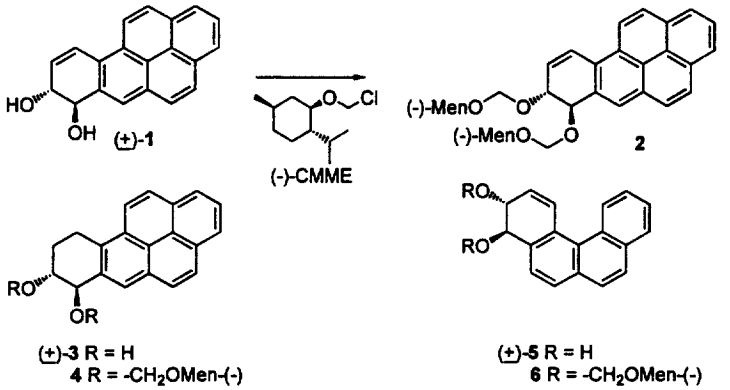
Racemic **1** was synthesized through known methods[9] and converted to the diastereomeric formaldehyde acetal mixture **2** (75–80%).[10] The corresponding transformation was also accomplished on the tetrahydrodiol (±)-**3** (Scheme 1). HPLC analysis (Axxiom silica 5 μ, 1×25 cm, eluted with 1.5% EtOAc–hexane at 4.5 mL/min) indicated that the dihydrodiol MOME derivatives (**2**) were marginally better separated than the tetrahydro analogs (**4**) ($\alpha_{dihydro}$=1.16, $\alpha_{tetrahydro}$=1.13), with baseline separation being achieved in both cases. Thus, preparative HPLC separation of ~550 mg of mixture **2** on the Axxiom column using 1.75% EtOAc–hexane, at a flow-rate of 3 mL/min afforded 263 mg of the *early*-isomer t_R=23.7 min, $[\alpha]_D$=−204.4 (*c*=4.5 mg/mL, THF), and 285 mg of the *late*-isomer t_R=26.2 min, $[\alpha]_D$=+79.8 (*c*=4.5 mg/mL, THF) [Figure 1(a)].[11] Reanalysis of the diastereomers indicated each to be >95% pure.



Scheme 1.

At this stage access to the dihydrodiol enantiomers only needed cleavage of the menthyloxymethyl moiety. After exploring several alternatives, we discovered that this could be smoothly accomplished within 20 min in AcOH containing a trace of 6 N HCl.[12] Since a highly labile species such as BaP dihydrodiol survives the deprotection conditions, we believe this procedure can be readily applied to compounds which are equally or less sensitive. Finally, the absolute configurations of the dihydrodiols

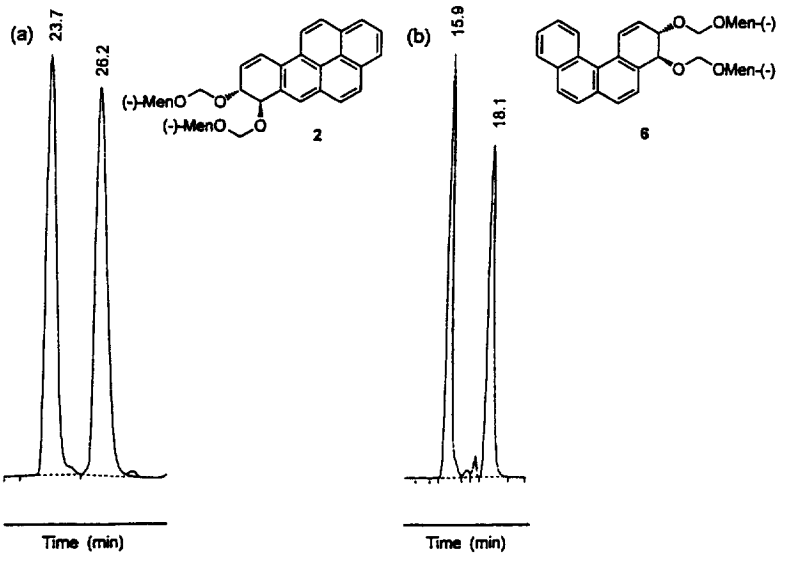


Figure 1. Analytical HPLC separation of the *bis-O*-MOME ethers **2** and **6**.

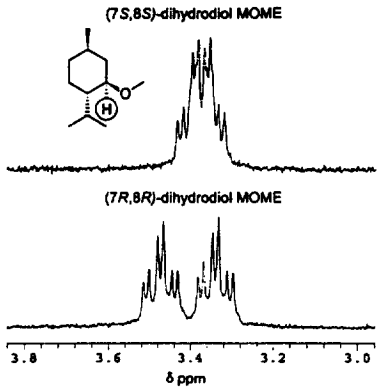


Figure 2. The C–*H*–O– proton resonances of the (7*R*,8*R*) and (7*S*,8*S*) BaP dihydrodiol bis-MOME derivatives.

were ascertained by optical rotation measurements and by comparison with the literature values.[2] The *early*-eluting diastereomer provides the (−)-(7*R*,8*R*) dihydrodiol, $[\alpha]_D$=−342.0 (*c*=1 mg/mL, THF), whereas the *late*-eluting isomer affords the (+)-(7*S*,8*S*) diol, $[\alpha]_D$=+367.0 (*c*=1 mg/mL, THF). Reverse-phase HPLC analysis (Beckman ODS 5 μ, 1×25 cm, eluted with 4:1 MeOH–H_2O, at 3 mL/min) indicated the chemical purity of each dihydrodiol to be >98%. Among the tetrahydrodiol MOME derivatives **4**, the (7*S*,8*S*) diol elutes before the (7*R*,8*R*) isomer.

There are interesting differences in the ^{1}H NMR spectra of the dihydrodiol MOME diastereomers. Significantly, in both $CDCl_3$ and C_6D_6, the –C*H*–O– proton of each menthyl moiety appears separately in the (7*R*,8*R*) isomer ($\Delta\delta$=0.13 ppm), whereas in the (7*S*,8*S*) case these show greater chemical shift equivalence (Figure 2, spectra in $CDCl_3$ at 300 MHz). This difference may prove to be diagnostic in the assignment of absolute configurations to other hydrocarbon dihydrodiol menthyloxymethyl ethers.

To test the generality of (−)-CMME as a resolving agent, we have prepared the MOME derivatives of the distorted benzo[*c*]phenanthrene dihydrodiol (±)-**5** (Scheme 1). In this case, HPLC analysis (Axxiom silica column eluted with 1.75% EtOAc–hexane, at a flow-rate of 5 mL/min) shows an even better separation [Figure 1(b), α=1.21] compared to the BaP derivatives: *early*-eluting **6** t_R=15.9 min, $[\alpha]_D$=−188.2 (*c*=5 mg/mL, THF); *late*-eluting **6** t_R=18.1 min, $[\alpha]_D$=+95.8 (*c*=5 mg/mL, THF). Initial NMR experiments reveal that the –C*H*–O– resonances of *late*-eluting **6** are simpler in appearance than those of the *early*-eluting isomer, which parallels the observation in the BaP case.

Use of (+)-CMME rather than the (−)-isomer reverses the elution order of the BaP dihydro and tetrahydrodiols as evidenced by the optical rotation data:[11] *early*-eluting dihydrodiol MOME $[\alpha]_D$=+202.2 (*c*=4.5 mg/mL, THF) and for the *late*-eluting isomer $[\alpha]_D$=−78.0 (*c*=4.5 mg/mL, THF), *early*-eluting tetrahydrodiol MOME $[\alpha]_D$=+8.2 (*c*=4.25 mg/mL, THF) and for the *late*-eluting isomer $[\alpha]_D$=+74.2 (*c*=4.5 mg/mL, THF).

Facile preparation of the MOME derivatives, greatly enhanced separations and the numerous methods available for cleavage of formaldehyde acetals makes this a convenient approach for enantiomer separation. This technique has obvious applications to the resolution of a wide variety of compounds and should be particularly useful in cases where the base-labile ester methodology cannot be applied.

Acknowledgements

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10. *Representative procedure for the preparation of the dihydrodiol MOME derivatives*: to a cooled, stirred solution of (±)-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene **1** (1.15 g, 4.0 mmol) in anhydrous DMF (15 mL) was added *N,N*-diisopropylethylamine (4.25 mL, 24.4 mmol) followed by (−)-CMME (5.0 g, 24.4 mmol). The mixture was flushed with argon and heated at 55–60°C for 6 h. The mixture was cooled, diluted with Et_2O and washed with H_2O. The organic layer was dried over Na_2SO_4 and evaporated. Chromatography of the crude product over silica gel using 2% EtOAc in hexane afforded the diastereomeric mixture **2** as a light yellow foam. This material was directly subjected to HPLC in 10–12 mg aliquots.
11. Under these conditions $\alpha_{dihydro}$=1.13, $\alpha_{tetrahydro}$=1.11. *Early*-eluting **4**: t_R=22.4 min, $[\alpha]_D$=−11.3 (*c*=4.5 mg/mL, THF), *late*-eluting **4**: t_R=24.4 min, $[\alpha]_D$=−71.1 (*c*=4.5 mg/mL, THF).
12. *Representative deprotection procedure*: to a mixture of the *late*-eluting isomer **2** (285 mg, 0.46 mmol) and AcOH (3.8 mL) was added 6 N HCl (26.6 μL). After 20 min at rt the reaction was complete as observed by TLC and precipitation of the product had occurred. The product was dissolved in 1:1 THF–EtOAc and the mixture was carefully washed to neutrality with sat. aq. $NaHCO_3$. The organic phase was dried over Na_2SO_4 and evaporated. Purification of the product either by trituration with Et_2O–acetone or by chromatography (silica gel, 1:1 THF–hexane) afforded the dihydrodiol (84 mg, 0.29 mmol) in 64% yield.

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