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Studies toward the Synthesis of 3,7β,8α-Trihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, A Reactive Metabolite of Benzo[a]pyrene

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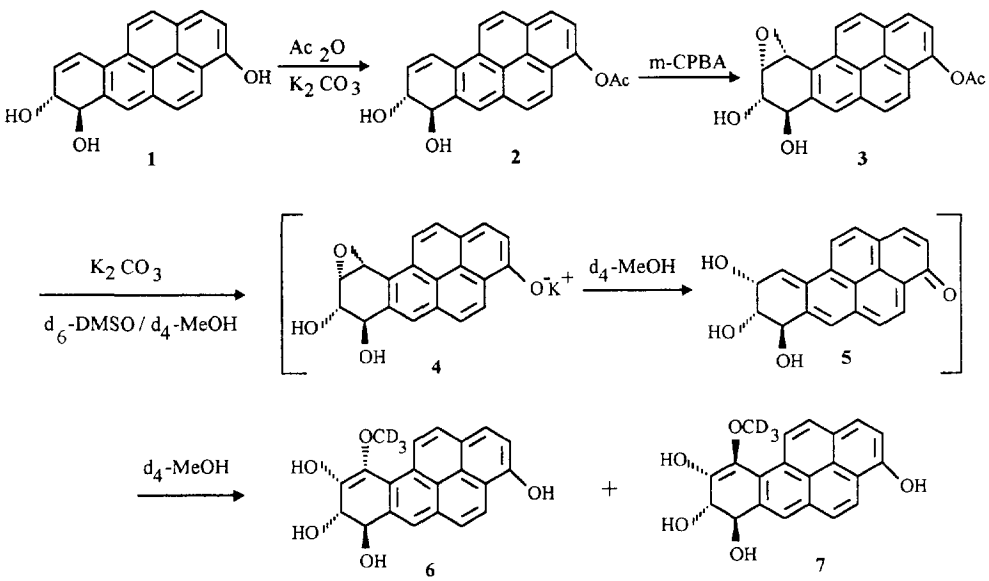
Abstract: Synthesis of 3,7β,8α-Trihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene by saponification of its 3-acetoxy analogue and by oxidation of 3,7α,8β-trihydroxy-7,8-dihydrobenzo[a]pyrene with dimethyldioxirane is investigated.

3,7β,8α-Trihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene(3-hydroxy-*anti*-BPDE)(**9**)has been characterized as a major reactive metabolite of 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (*anti*-BPDE),[1] a putative ultimate carcinogen of the most widely studied environmental carcinogen benzo[a]pyrene (BP).[2] The participation of the phenolic group of 3-Hydroxy-*anti*-BPDE for additional stabilization of the transient carbonium ion produced at the C-10 benzylic carbon renders 3-hydroxy-*anti*-BPDE theoretically more electrophilic; consequently, 3-hydroxy-*anti*-BPDE is postulated to be more carcinogenic than *anti*-BPDE.[3] Despite its biological importance, methods for the synthesis of 3-hydroxy-*anti*-BPDE have not been available. The standard procedure,[4,5] which requires the stereoselective epoxidation of the dihydrodiol with m-chloroperoxybenzoic acid (m-CPBA) was not applicable to the synthesis of 3-hydroxy-*anti*-BPDE.[6] Therefore, an alternate method for the synthesis of 3-hydroxy-*anti*-BPDE was urgently needed so that its importance in the metabolic activation of BP could be probed. In an earlier study[6] the synthesis of 3-trimethylacetyl-*anti*-BPDE was reported in high yield; however, the hydrolytic cleavage of the sterically bulky ester group of 3-trimethylacetyl-*anti*-BPDE was always accompanied by the destruction of the epoxide functionality. It has been previously reported[4,7] that dry K_2CO_3 in absolute CH_3OH can cleave the relatively labile acetyl protecting group of some acetylated diol epoxides or phenolic diol epoxides without destroying the epoxide functionality. These reports and the personal observation that *anti*-BPDE is sufficiently stable in K_2CO_3/MeOH prompted me to study the synthesis of 3-acetoxy-*anti*-BPDE, and its deacetylation with K_2CO_3/MeOH as one of the possible methods for the synthesis of 3-hydroxy-*anti*-BPDE. The key starting material, 3-hydroxy BP-7,8-*trans*-dihydrodiol (**1**),[8] was stirred vigorously with 50% molar excess of Ac_2O in dry acetone in the presence of freshly ignited K_2CO_3 at 0 °C for 15 min (Scheme 1). The major relatively polar product, which was isolated by extraction in 83% yield, was characterized as 3-acetoxy-BP-7,8-*trans*-dihydrodiol (**2**),[9] mp 203-205 °C. The exact mass (M^+, 344.1026) and the molecular formula ($C_{22}H_{16}O_4$), obtained by the high resolution mass spectrum of **2**, suggested that only one of the three hydroxyl groups of triol **1** was acetylated. The observation that chemical shift and coupling constants (J values)

Scheme I

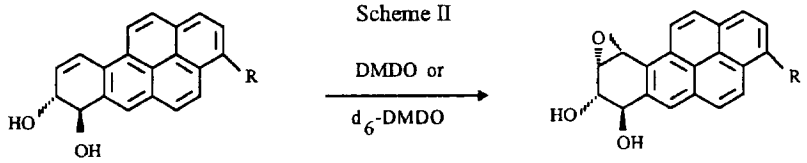


involving methine protons H_7, H_8, H_9, and H_{10} of **2** (Table) were nearly identical to those of the analogous protons of triol **1**[10] confirmed that alcoholic hydroxyl groups of **2** were not acetylated.

The stereoselective conversion of **2** to (±)-3-acetoxy-*anti*-BPDE (**3**) was readily achieved by stirring **2** with 16 molar equivalent of purified m-CPBA in dry THF ($LiAlH_4$) for 15 min at room temperature under argon. The modification of the workup procedure, which required extraction of the ice-cold ethyl acetate solution of the reaction mixture with cold 5% solution of sodium sulfite, followed by 10% aqueous sodium bicarbonate and water gave, pure **3** (mp 186-188 °C, dec) in 94% yield. The deacetylation of 3-acetoxy-*anti*-BPDE (**3**) with K_2CO_3 was examined *in situ* by ^{1}H- NMR in a deuterated solvent. In a typical experiment, ^{1}H-NMR spectra of **3** (~ 1.0 -1.5 mg) in 500 μl of 10% d_6-DMSO in d_4-methanol were taken before and after shaking the solution with anhydrous K_2CO_3 (0.5 - 1 mg) at 0 °C for 1-2 min. The ^{1}H-NMR spectrum of the resulting light yellow solution of the product (Table) indicated a complete disappearance of the acetoxy proton signal (δ 2.55 ppm) from **3**, and the formation of two major products identified on the basis of coupling constants and decoupling experiments as 3,7β,8α,9α-tetrahydroxy-10α-methoxy-7,8,9,10-tetrahydroBP (**6**) (~ 75%) and 3,7β,8α,9α-tetrahydroxy-10β-methoxy-7,8,9,10-tetrahydroBP (**7**) (~ 25%). No evidence for the presence of 3-hydroxy-*anti*-BPDE (**9**) (see Scheme II for structure) as a major product was noted. A rapid formation of **6** and **7** only after a mild treatment of **3** with K_2CO_3 suggests that because of the sufficient acidity of the phenolic group, the resulting triol **9** readily forms a highly electrophilic species, phenolate ion **4** or isomerized quinone methide **5**,[3] in K_2CO_3/MeOH (Scheme I).

It appeared from the above studies that 3-hydroxy-*anti*-BPDE (**9**) does not survive under acidic (m-CPBA or m-CBA) or basic (K_2CO_3/MeOH) reaction conditions during its synthesis. Therefore, we extended

our investigation to the application of dimethyldioxirane (DMDO)[11] as a potential reagent for the epoxidation of triol **1** to its epoxide **9**. The reaction of DMDO takes place under mild and strictly neutral conditions, and is considered to be the reagent of choice for epoxidizing electron rich alkenes whose epoxides are usually too labile for isolation and even spectral detection.[11-13] The previously reported use of DMDO in the synthesis of polynuclear aromatic hydrocarbon derivatives has been limited to only a few K-region oxides[11] and non-bay-region diol epoxides[14]; its use in the synthesis of bay-region diol epoxides has not been reported. Although a brief treatment (1 - 2 min) of BP 7,8-*trans*-dihydrodiol (**8**) or its 3-acetoxy derivative **2** with DMDO[12] (Scheme II) and evaporation of the solvent at 0 °C cleanly produced a ~ 1:3 mixture of the corresponding bay-region *syn*- and *anti*-diol epoxides (^{1}H-NMR), a similar treatment of the triol **1** with DMDO always resulted in the isolation of a dark red product of polymeric nature. However, when the epoxidation of triol **1** was carried out with a 0.1 M solution of d_6-DMDO[15] in d_6-acetone in an NMR tube,



1: R = OH; **2**: R = OAc; **8**: R = H

9: R = OH; **3**: R = OAc; **10** R = H

and the product was immediately analyzed *in situ* by ^{1}H-NMR, the formation of one major compound (~ 85%) whose ^{1}H-NMR (Table) was entirely consistent with the structure of **9** was observed. *syn*-Diol epoxide was also detected in a minor amount (~ 15%). The NMR sample which deteriorated significantly within 15 min at room temperature was sufficiently stable at -78 °C. Presumably, enhanced interaction of the phenolic group with the reactive 9,10-epoxide is responsible for the polymerization of **9** during its isolation.

Table 400 MHz ^{1}H-NMR spectrum of benzo[a]pyrene derivatives synthesized in the present study

Compound	Methine Protons				OAc	Aromatic Protons
	H-7	H-8	H-9	H-10		
2	4.90	4.43	6.23	7.52	2.52	7.80-8.50
(DMSO-d_6 + MeOH-d_4)	($J_{7,8}$ = 10.8; $J_{8,9}$ = 0; $J_{9,10}$ = 10.7)					
3	4.91	4.04	3.95	5.19	2.55	7.80-8.70
(10% DMSO-d_6 in MeOH-d_4)	($J_{7,8}$ = 8.8; $J_{8,9}$ = 0; $J_{9,10}$ = 4.9)					
6	5.05	3.86	4.41	5.17		7.12-8.36
(10% DMSO-d_6 in MeOH-d_4)	($J_{7,8}$ = 4.9; $J_{8,9}$ = 2.9; $J_{9,10}$ = 3.9)					
7	4.90	3.96	4.48	4.90		7.14-8.36
(10% DMSO-d_6 in MeOH-d_4)	($J_{7,8}$ = 8.8; $J_{8,9}$ = $J_{9,10}$ = 1.9)					
9	4.92	4.04	3.89	5.08		7.59-8.45
(Acetone-d_6 + MeOH-d_4)	($J_{7,8}$ = 8.6; $J_{8,9}$ = 0; $J_{9,10}$ = 4.3)					

The present study demonstrated the first feasible approach for the synthesis of labile 3-hydroxy-*anti*-BPDE. This study, in combination with a previous study[14], also demonstrated that the stereoselectivity of DMDO in the epoxidation of dihydrodiol is highly dependent upon the conformation of the vicinal diols. Dihydrodiols having vicinal diols in *quasi*-diaxial conformation (e. g. bay-region dihydrodiols) are epoxidized by DMDO to *syn*-diol epoxides. In contrast, dihydrodiols having vicinal diols in *quasi*-diequatorial conformation (e. g. non-bay-region dihydrodiols) are epoxidized predominantly to *anti*-diol epoxides.

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