Stereochemistry of the Hydrolysis Products and Their Acetonides of Two Stereoisomeric Benzo[a]pyrene 7,8-Diol 9,10-Epoxides

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Abstract: Two stereoisomeric benzo[a]pyrene diol epoxides, *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene (I) and *r*-7, *t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene (II), have each been found to rapidly hydrolyze in water to a pair of stereoisomeric tetrahydroxytetrahydrobenzo[a]pyrenes (tetrols). The stereoisomeric tetrols have been resolved by high-pressure liquid chromatography. The stereochemistry of the tetrols has been established unequivocally by ultraviolet absorption spectra, reactions with potassium triacetylosmate, and mass spectra of the tetrols and their cis 1,2-monoacetonides and cis 1,3-diacetonide which were resolved by high-pressure liquid chromatography. Product analyses indicate that the tetrols are formed by initial cleavage of the C(10)–O bond of the diol epoxides through carbonium ion intermediates at C(10). Nucleophilic addition by water proceeds trans stereoselectively for I and cis stereoselectively for II both at C(10) position. The C(10) OH of (7,9/8,10)-tetrol, which is derived from II, is labile. Thus the C(10) OH of this tetrol is exchangeable with solvent water molecules in acidic oxygen-18 water/tetrahydrofuran solution to yield a major (7,9,10/8)-tetrol and a minor (7,9/8,10)-tetrol, both containing oxygen-18. In addition this tetrol forms a cis 9,10-acetonide as a result of the labile C(10) OH of the tetrol which is catalyzed by anhydrous copper sulfate in acetone.

There is growing evidence that benzo[a]pyrene 7,8-diol 9,10-epoxides[1c] are metabolites responsible for the carcinogenic action of benzo[a]pyrene, a widespread environmental pollutant which may be causal in human cancer. The diol epoxides are strongly mutagenic[2-6] and bind to DNA and RNA in vitro and mammalian cells in culture,[7-10] and are chemically very reactive and unstable in aqueous media.[2,12] Their characterization in biological systems can be accomplished by the detection of their hydrolysis products, the tetrols.[2,12] This paper reports the elucidation of the stereochemistry of the tetrols derived from two synthetic[11,13,14] BP 7,8-diol 9,10-epoxides. The modes of acetonide formation of tetrols were explored and the mass spectra of these derivatives characterized.

In metabolic studies, diol epoxide I was found to be the predominant diol epoxide formed and a far more potent mutagen than the isomeric synthetic diol epoxide II, K-region 4,5-epoxide, or any other known BP derivatives[2,12] It was reported subsequently that a single enantiomer of diol epoxide I is highly stereoselectively formed by the rat liver microsomal mixed-function oxidases[12] and cultured human bronchus[15] from the metabolically formed and optically pure (−)-*r*-7,*t*-8-dihydroxy-7,8-dihydrobenzo[a]pyrene ((−)-*trans*-7,8-diol).[12,16] The metabolic formation of diol epoxides from (−)-*trans*-7,8-diol was demonstrated by the detection of their hydrolysis products (tetrols)[2,12,16,17] and NADPH or NADH reduction products (triols).[12,18]

The high hydrolytic reactivity of the two stereoisomeric diol epoxides prompted us to investigate the mechanism of hydrolysis by characterization of the reaction products. The results indicate that carbonium ions formed at C(10) are the intermediates in the hydrolysis of both diol epoxides I and II.

Experimental Section

Materials. Synthetic BP derivatives were obtained through National Cancer Institute Contract N01-CP-33387[13,19] and N01-CP-33385.[14] Information on the availability of the compounds can be obtained from the Manager, Information and Resources Segment, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. 20014. Potassium triacetylosmate was synthesized according to the procedure of Criegee et al.[20]

Preparation of Tetrols. One milligram of I or II (in 0.2 mL of tetrahydrofuran) was treated with water (200 mL) at room temperature for 24 h. The hydrolysis products (tetrols) were extracted twice with 200 mL of ethyl acetate. After dehydration with anhydrous magnesium sulfate the ethyl acetate was evaporated to dryness under reduced pressure. The tetrols were isolated by high-pressure liquid chromatography.

Preparation of Acetonides. Each of the HPLC resolved tetrols was dried in a vacuum desiccator over KOH overnight. Anhydrous acetone was added to dissolve the tetrols and a catalytic amount of anhydrous copper sulfate was then added.[21] The solution was allowed to stand at room temperature for at least 4 h with agitation. The acetone solution was evaporated to dryness and 1 drop of pyridine and 2 drops of tetrahydrofuran were added to dissolve the acetonides for HPLC analysis.

High-Pressure Liquid Chromatography. A Spectra-Physics Model 3500 liquid chromatograph fitted with a Du Pont 6.2 mm i.d. × 0.25 m "Zorbax" octadecyltrimethoxysilane (ODS) column was used. Tetrols were resolved with 65% methanol in water as the eluent. The acetonides of tetrols were isolated with a linear gradient of 65% methanol in water to 100% methanol for 30 min. Owing to the gradual deterioration of column separation efficiency, several ODS columns were used during the course of this investigation. Retention times of compounds among different sets of experiments are therefore not to be extrapolated. All HPLC were carried out at room temperature at a solvent flow rate of 0.8 mL/min.

Mass Spectra. The mass spectra were performed on a JEOL JMS-01SG-2 instrument at 70 eV ionizing voltage. The samples in tetrahydrofuran or acetone were evaporated onto the solid probe for mass spectral determinations. High-resolution data were collected using Ilford Q-2 photographic plates. Mass spectra of tetrols were recorded at 240 °C probe temperature and at approximately 350 °C ion source temperature. The relative intensities of major ions of tetrol I-1 are *m/e* 320 (100, $C_{20}H_{16}O_4$), 302 (25), 284 (44), 271 (17), 268 (18), 260 (20, $C_{18}H_{12}O_2$), 256 (49), 255 (63, $C_{19}H_{11}O$), 242 (28), 239 (24), 231 (33), 226 (33), 215 (48), 214 (36), 213 (29), 203 (73), 202 (64), 201 (32), 200 (21), and 113 (41). The mass spectra of all other tetrols are qualitatively similar to that of I-1. The *m/e* 320 to *m/e* 284 peak intensity ratios are temperature dependent.

Mass Spectral Analysis for ^{18}O. I and II were each hydrolyzed in $^{18}OH_2$ (95 atom %, Bio-Rad). Mass spectral analysis of all four HPLC-resolved tetrols indicate the ^{18}O content, calculated from the ratio of (M + 2)/M + (M + 2), to be 90–95 atom %. The mass spectral analysis indicates that the ^{18}O of the acetonides derived from the ^{18}O-labeled tetrols I-1 and I-2 were completely retained. Only 65% retention of the ^{18}O was found in the acetonide derived from ^{18}O-labeled tetrol II-2. However, no ^{18}O was detected by mass spectral

analysis of the HPLC-resolved monoacetonide and diacetonide which were derived from the ^{18}O-labeled tetrol II-1. These observations suggest that the C(10) OH[22] of tetrols II-1 and II-2 are labile; the degree of lability is more extensive in tetrol II-1. The relative intensities of major ions of ^{18}O-labeled tetrol I-1 are *m/e* 322 (57), 320 (6), 304 (8), 302 (7), 286 (15), 284 (21), 273 (9), 271 (15), 268 (8), 262 (15), 258 (26), 257 (40), 256 (39), 255 (46), 244 (21), 239 (21), 231 (39), 226 (46), 215 (61), 214 (55), 213 (52), 203 (100), 202 (93), 201 (45), and 200 (35). The major ions of other ^{18}O-labeled tetrols are qualitatively similar to that of the ^{18}O-labeled tetrol I-1.

For the monoacetonide of ^{18}O-labeled tetrol I-1: *m/e* 362 (92), 360 (18), 347 (4), 344 (4), 342 (3), 329 (3), 327 (2), 304 (4), 287 (15), 286 (11), 285 (12), 284 (10), 274 (11), 271 (13), 268 (10), 262 (19), 258 (33), 257 (88), 256 (59), 255 (100), 244 (15), 242 (17), 239 (39), 231 (28), 226 (52), 215 (40), 203 (52), 202 (68), 201 (36), and 200 (19). For the monoacetonide 1 (retention time 23.1 min on HPLC) of ^{18}O-labeled tetrol I-2: *m/e* 362 (100), 360 (10), 347 (2), 344 (2), 329 (7), 304 (4), 287 (12), 286 (12), 285 (12), 284 (29), 274 (18), 271 (15), 268 (7), 262 (16), 258 (35), 257 (74), 256 (59), 255 (70), 244 (14), 239 (31), 231 (25), 226 (43), 215 (34), 203 (51), 202 (63), 201 (33), and 200 (18). For the monoacetonide 2 (retention time 25.3 min on HPLC) of ^{18}O-labeled tetrol I-2: *m/e* 362 (100), 360 (12), 347 (2), 344 (2), 329 (4), 304 (2), 302 (3), 286 (18), 285 (58), 284 (41), 268 (9), 257 (34), 256 (48), 255 (48), 242 (11), 239 (34), 232 (98), 231 (65), 226 (41), 215 (44), 203 (91), 202 (49), 201 (23), and 200 (13). For the monoacetonide of ^{18}O-labeled tetrol II-2: *m/e* 362 (74), 360 (47), 347 (1), 345 (1), 344 (1), 342 (1), 329 (1), 327 (1), 302 (3), 285 (100), 257 (34), 256 (30), 255 (50), 242 (13), 239 (40), 232 (85), 231 (55), 226 (51), 215 (54), 203 (84), 202 (59), 201 (27), and 200 (16).

Mass Spectral Analysis for ^{14}C. BP [7-^{14}C]diol epoxide I (specific activity 53.9 mCi/mmol)[23] was hydrolyzed in water and tetrols I-1 and I-2 were separated on HPLC. The relative intensities of major ions of ^{14}C-labeled tetrol I-1 are *m/e* 322 (77), 320 (8), 304 (22), 286 (50), 273 (23), 270 (15), 262 (19), 258 (58), 257 (86), 244 (29), 241 (20), 239 (22), 233 (35), 228 (31), 226 (34), 217 (36), 215 (54), 203 (100), 202 (93), 201 (49), and 200 (34). The major ions of ^{14}C-labeled tetrol I-2 are qualitatively similar to those of tetrol I-1.

Acetonides were made from the ^{14}C-labeled tetrols I-1 and I-2 and purified by HPLC. The relative intensities of major ions of the monoacetonide of the ^{14}C-labeled tetrol I-1 are *m/e* 362 (75), 360 (6), 347 (4), 344 (2), 329 (4), 304 (4), 287 (16), 286 (11), 276 (8), 273 (13), 270 (7), 262 (15), 258 (51), 257 (100), 244 (16), 241 (19), 239 (14), 233 (15), 231 (11), 228 (26), 226 (26), 217 (13), 215 (22), 203 (37), 202 (50), 201 (23), and 200 (15). For the monoacetonide 1 of the tetrol I-2: *m/e* 362 (75), 360 (7), 347 (1), 329 (6), 304 (3), 287 (17), 286 (31), 276 (21), 273 (15), 270 (7), 262 (13), 258 (69), 257 (100), 244 (14), 241 (22), 239 (19), 233 (17), 231 (13), 228 (30), 226 (30), 217 (17), 215 (28), 203 (48), 202 (63), 201 (34), and 200 (19). For the monoacetonide 2 of the tetrol I-2: *m/e* 362 (100), 360 (9), 347 (2), 344 (1), 329 (3), 304 (2), 287 (51), 286 (27), 270 (8), 258 (46), 257 (52), 241 (24), 239 (16), 234 (81), 233 (58), 228 (23), 226 (32), 217 (14), 215 (39), 203 (88), 202 (52), 201 (24), and 200 (14).

Copper Sulfate Catalyzed Isomerization of II-1 to II-2 in Anhydrous THF. HPLC isolated tetrol II-1 (20 μg) was dried and dissolved in 1 mL of anhydrous THF and ca. 0.1 mg of anhydrous copper sulfate was added. The mixture was allowed to stand at room temperature overnight and the THF was removed and evaporated to dryness. The residue was redissolved in 50 μL of THF/H_2O (1:1 v/v). Both tetrol II-1 (ca. 95%) and tetrol II-2 (ca. 5%) were found by HPLC analysis. However, tetrol II-2 was not found when tetrol II-1 was dissolved in anhydrous THF alone.

Acid-Catalyzed Exchange of C(10) OH of Tetrol II-1. Unlabeled tetrol II-1 (20 μg, purified by HPLC) was dissolved in 0.7 mL of THF/oxygen-18 water (1:3 v/v; oxygen-18 water, 95 atom %, Bio-Rad) and 0.09 N in HCl. The solution was allowed to stand at room temperature for 4 days and was then evaporated to dryness under nitrogen after neutralization with NaOH. The residue was dissolved in 50 μL of THF/water (1:1 v/v) and tetrols were isolated by HPLC. Both tetrols II-1 (13%) and II-2 (87%) were recovered. The recovered tetrols II-1 and II-2 were found by mass spectral analysis to contain 38 and 88% oxygen-18, respectively. About 70% of tetrol II-1 was converted to tetrol II-2 when the above acid-catalyzed reaction was carried out at room temperature for 1 h.

Acid Hydrolysis of the Monoacetonides of Tetrols II-1 and II-2. The HPLC resolved monoacetonides of tetrols II-1 (ca. 5 μg) and of tetrol II-2 (ca. 5 μg) were dissolved in 1 mL of THF/water (1:1 v/v) and 0.09 N in HCl. The solution was allowed to stand at room temperature for 1 h and was then neutralized with NaOH. The solution was extracted with 2 × 2 mL of ethyl acetate. The ethyl acetate extract was then evaporated and the residue redissolved in 25 μL of THF. For either sample, only tetrol II-2 was found by HPLC analysis.

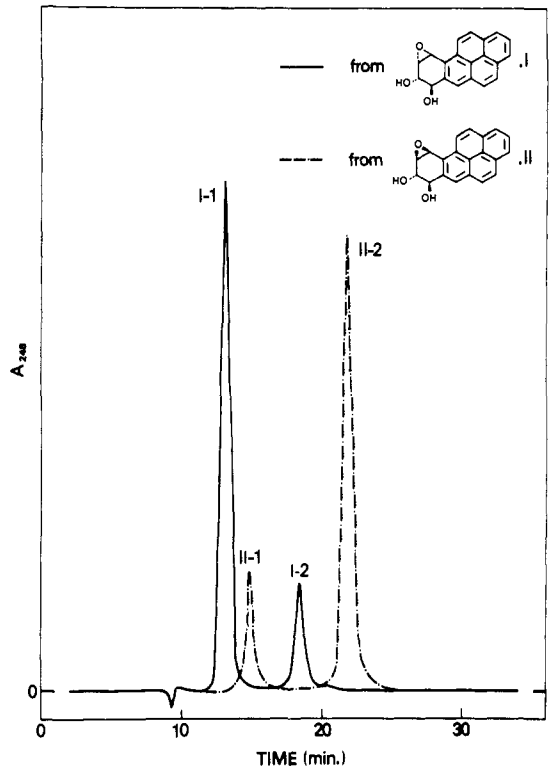


Figure 1. HPLC separation of the hydrolysis products of benzo[*a*]pyrene diol epoxide I (solid curve) and diol epoxide II (dotted curve).

Results

HPLC Separation and Mass Spectra of the Hydrolysis Products of Diol Epoxides I and II. The two stereoisomeric BP diol epoxides undergo rapid hydrolysis in aqueous media.[2,12] Diol epoxides I and II each give two products and the resulting four compounds are distinct and separable on HPLC (Figure 1). The ultraviolet absorption spectra indicated that all four isolated compounds have saturated carbons at 7,8,9,10 positions of BP.[2,12] The low- and high-resolution mass spectral analyses establish unequivocally that the hydrolysis products of the two stereoisomeric diol epoxides are all 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenes (tetrols).

Vicinal Cis Diol Test. Potassium triacetylosmate reacts irreversibly with compounds containing vicinal cis diol and the color of the reaction mixture changes from blue to yellow.[20,24] The results shown in Table I indicate that only tetrol II-1 (Figure 1) gave a negative response, which suggests that the four neighboring hydroxyl groups of tetrol II-1 are all trans to each other.

HPLC Separation and Mass Spectra of the Tetrol Acetonides. The isolated tetrols of I and II were each reacted with acetone in the presence of anhydrous copper sulfate.[21] Initial mass spectral analyses showed that the acetonides of all four tetrols have molecular ions at *m/e* 360 indicating the formation of monoacetonides. Only the spectrum of derivatized tetrol II-1 indicated the presence of a trace amount of diacetonide, with a molecular ion at *m/e* 400. We have developed HPLC conditions which separate the monoacetonide from the diacetonide (Figure 2). This HPLC method not only separates the monoacetonide from the diacetonide, but also separates the isomeric monoacetonides (Figure 2). Both the monoacetonides and the diacetonide are stable under the HPLC conditions.

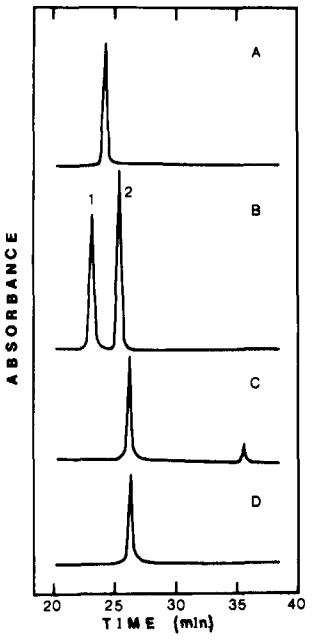


Figure 2. HPLC separation of mono- and diacetonides of benzo[*a*]pyrene tetrols. A, from tetrol I-1, t_R 24.1 min; B, from tetrol I-2, t_R 23.1 and 25.3 min, respectively; C, from tetrol II-1, t_R 26.3 and 35.7 min, respectively; D, from tetrol II-2, t_R 26.3 min. The tetrols were isolated as shown in Figure 1.

Tetrol I-2 and tetrol II-1 both gave two HPLC separable acetonides (Figure 2). Mass spectral analyses (Figure 3) indicate that both acetonides of tetrol I-2 are monoacetonides (45 and 55%, respectively). Tetrol II-1 yields a major monoacetonide (ca. 90%) and a minor diacetonide (ca. 10%). Only one monoacetonide was found to form from either tetrol I-1 or tetrol II-2.

Lability of C(10) OH of Tetrol II-1. The ^{18}O-labeled tetrol II-1 loses its label upon acetonation, which suggests that the C(10) OH is labile. In acidic $^{18}OH_2$/THF solution the unlabeled tetrol II-1 is converted to both tetrols II-1 and II-2, but predominantly to the latter. Both tetrols were found by mass spectral analysis to contain ^{18}O. The above results thus establish that the C(10) OH of tetrol II-1 is labile and exchangeable with solvent water molecules. Whereas partial loss of ^{18}O was observed for ^{18}O-labeled tetrol II-2 upon acetonation, the ^{18}O of ^{18}O-labeled tetrol II-1 was completely lost upon acetonide formation. The results thus indicate that the C(10) OH is also labile in anhydrous acetone containing copper sulfate. The formation of unlabeled acetonide from ^{18}O-labeled tetrol II-1 thus suggests that the C(10) oxygen of the acetonide is derived from acetone.

The Monoacetonides of Tetrols II-1 and II-2 Are Identical. The retention times on HPLC and the mass spectra of the monoacetonides of tetrols II-1 and II-2 are identical, which suggest that the structures of the monoacetonides are identical. When the monoacetonides of both tetrols II-1 and II-2 were hydrolyzed in acidic solution, only tetrol II-2 was found by HPLC analysis. These results thus furnish strong evidence that the cis 9,10-monoacetonide of tetrol II-1 is formed by first losing the C(10) OH followed by reaction with acetone.

Discussion

Since the stereochemistry of I and II is shown,[11,14] there are only three possible stereoisomeric tetrols (Figure 4) which could be formed from each of the two stereoisomeric diol epoxides. This is deduced by the consideration that the 9,10-epoxide ring can be cleaved at either C(10) or C(9). HPLC and mass spectral analyses indicated that only two tetrols were formed from either I or II, and none of the tetrols have the same retention time on HPLC (Figure 1). Figure 4 indicates that (7,10/8,9)-tetrol and (7,9/8,10)-tetrol can be derived from either I or II. The (7/8,9,10)-tetrol can only be derived from I and the (7,9,10/8)-tetrol can only be derived from II. The structural assignments for the four tetrols and their acetonides are described below.

Table I. Vicinal Cis Diol Test with Potassium Triacetylosmate[a]

BP derivative[b]	Obsd color	Vicinal cis diol containing
None	Light blue	
cis-4,5-Diol	Yellow	Yes
trans-4,5-Diol	Light green	No
cis-7,8-Diol	Yellow	Yes
trans-7,8-Diol	Light gray	No
trans-9,10-Diol	Light purple	No
cis-7,8-Tetrahydrodiol	Yellow	Yes
trans-7,8-Tetrahydrodiol	Greenish yellow	No
cis-9,10-Tetrahydrodiol	Yellow	Yes
trans-9,10-Tetrahydrodiol	Greenish yellow	No
Tetrol I-1	Yellow	Yes
Tetrol I-2	Yellow	Yes
Tetrol II-1	Light green	No
Tetrol II-2	Yellow	Yes

[a] The tests were carried out with 25–50 μg of the compound indicated in 50 μL of glacial acetic acid/tetrahydrofuran (1:1 v/v) and 50 μL of 2 mM potassium triacetylosmate in glacial acetic acid. The colors recorded were those observed immediately after mixing. [b] Except for the tetrols, all other BP derivatives are synthetic standards.[13,19]

Assignment of Tetrol Structures. Tetrol II-1 Is (7,9/8,10)-Tetrol. The vicinal cis diol test with potassium triacetylosmate has been applied to a number of synthetic derivatives and has given consistently correct responses to compounds containing vicinal cis diols (Table I). Of the four tetrols, only II-1 gave a negative response, which suggests that the four neighboring hydroxyl groups are all trans to each other. Mass spectral analyses of HPLC resolved acetonides (Figures 2 and 3) indicate that only tetrol II-1 gave a diacetonide (M^+ *m/e* 400) in addition to a major monoacetonide (M^+ *m/e* 360).

The 1,3 mode of acetonide formation is only rarely observed, such as in the case of pyranosides,[26] where the acetonide is bridging the C(4) hydroxyl to the exocyclic C(6) hydroxymethyl group of the sugar molecule, or in bridging transannular diaxial hydroxyls, e.g., on the carbocyclic skeleton of hexahydrophenanthrene.[27] The mass spectrum of the diacetonide of tetrol II-1 (Figure 3) conforms well with the (7,9/8,10) configurations, exhibiting an intense molecular ion, and sequential losses of acetone (*m/e* 342), methyl radical (*m/e* 327), and ketene (*m/e* 285).[28] The formation of a diagnostic ion at *m/e* 272, which is absent in all other monoacetonides (Figure 3), could be visualized through ring fragmentation upon electron impact, as depicted in Figure 5A.

The above finding that a diacetonide is formed by bridging two pairs of hydroxyl groups in a cis 1,3 fashion which is consistent with the vicinal cis diol test (Table I) provide the cornerstone in the stereochemical elucidation of all the stereoisomeric tetrols and their acetonides.

It has been generally agreed that the acetonide derivatives of polyols on six-membered carbocyclic rings (cyclitols) or on more complex structures form only in a cis 1,2 fashion bridging the vicinal cis hydroxyl groups.[29] Only under forced conditions the cyclitols yield traces of trans 1,2-acetonide.[30] The introduction of unsaturation or fusion of an aromatic ring to a cyclitol skeleton may impart to the cyclohexane ring a conformational rigidity, and may thus alter its mode of reactivity toward acetonation reaction. The model compound, *trans*-

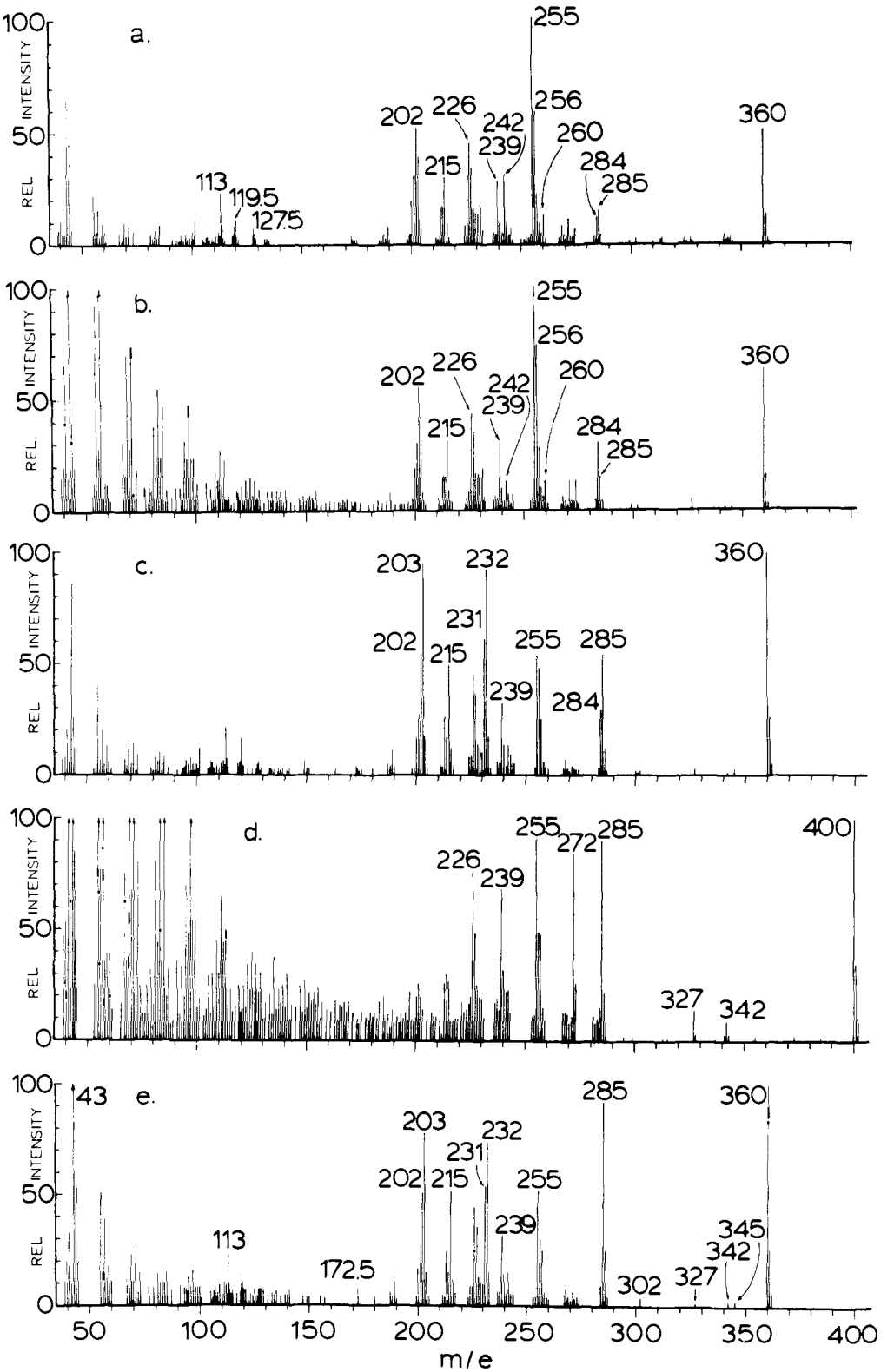


Figure 3. Mass spectra of HPLC resolved BP tetrol acetonides. The acetonides derived from tetrols were isolated as shown in Figure 2. They are derived from tetrol I-1 (a), acetonide 1 of tetrol I-2 (b), acetonide 2 of tetrol I-2 (c), diacetonide of tetrol II-1 (d), and monoacetonide of tetrol II-1 (e). The mass spectrum of the monoacetonide of tetrol II-2 is identical with that of the monoacetonide of tetrol II-1 (e).

7,8-dihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, did not form any trace of acetonide under our conditions.[31]

The structure of the major monoacetonide of tetrol II-1 (Figures 2 and 3) has been determined to be a cis 9,10-monoacetonide. This is demonstrated by the result that tetrol II-2 is the only product in the acid hydrolysis of the monoacetonide of tetrol II-1. The formation of cis 9,10-monoacetonide from tetrol II-1 is due to the lability of the C(10) OH. The C(10) OH is isomerized by the catalysis of copper sulfate in anhydrous THF solution to tetrol II-2. The lability of the C(10) OH of tetrol II-1 is further demonstrated by its exchangeability under acidic conditions with solvent water molecules to form predominantly tetrol II-2 and a minor amount of tetrol II-1.

The above results thus established unequivocally that (1) the hydroxyl groups of tetrol II-1 have (7,9/8,10) configurations, (2) the diacetonide of tetrol II-1 is formed by bridging

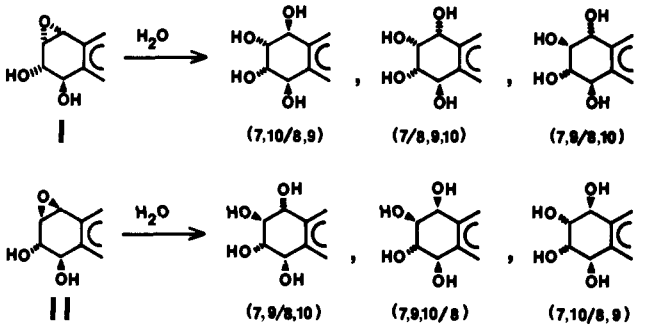


Figure 4. Structure of all possible hydrolysis products of BP diol epoxides I and II.

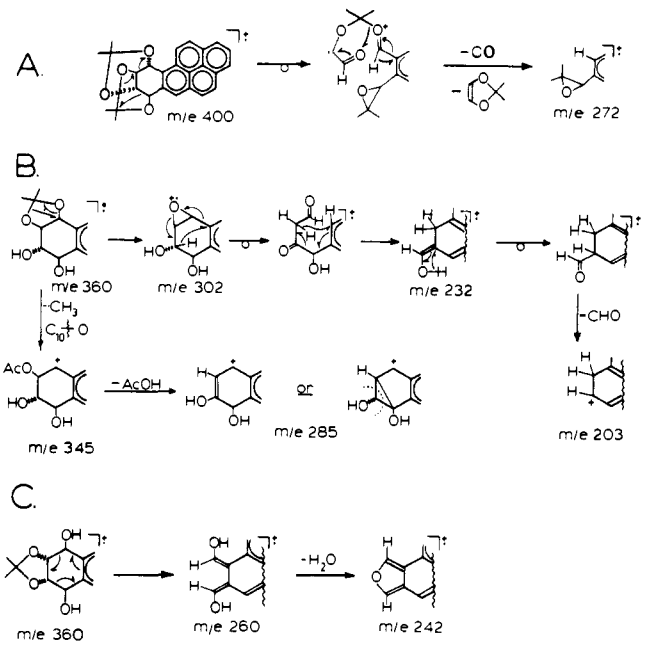


Figure 5. Proposed fragmentation pathways for the diagnostic ion formations in the mass spectra of (7,9/8,10)-diacetonide (A, from tetrol II-1), 9,10-monoacetonide (B, from tetrol II-2), and 8,9-monoacetonide (C, from tetrol I-1).

of cis 7,9 and cis 8,10 hydroxyls, and (3) the C(10) OH of tetrol II-1 is labile.

Tetrol II-2 Is (7,9,10/8)-Tetrol. Since we have established that tetrol II-1 is (7,9/8,10)-tetrol, according to the above considerations tetrol II-2 must have (7,9,10/8) configurations. The conclusion is supported by the acetonide formed from tetrol II-2 which is not derived from (7,10/8,9)-tetrol. The latter would be derived from the cleavage of C(9)–O bond of II.

Only one monoacetonide was found for tetrol II-2 (Figure 3) which has an identical mass spectrum and HPLC retention time with the tetrol II-1 monoacetonide (Figure 2). The acid hydrolysis of both the monoacetonides of tetrols II-1 and II-2 yield only tetrol II-2. The results thus establish that the structure of the monoacetonide of II-2 is identical with that of the monoacetonide of II-1. The 9,10-acetonide linkage is consistent with the mass spectrum. The formation of diagnostic ions, such as *m/e* 232 and 203, can be explained through concerted acetone loss, ring cleavage, and a tautomeric shift accompanied by favorable double and triple hydrogen transfers, respectively (Figure 5B). The consecutive losses of methyl radical and acetic acid from the molecular ion yield an intense ion at *m/e* 285, which on further loss of formaldehyde leads to *m/e* 255. Thus the results have established that tetrol II-2 has a (7,9,10/8) configuration.

Tetrol I-1 Is (7,10/8,9)-Tetrol and Tetrol I-2 Is (7/8,9,-10)-Tetrol. None of the tetrols from I or their acetonides overlap with those from II on HPLC (Figures 1 and 2). Therefore, (7,9/8,10)-tetrol, which has been identified as the structure for II-1, cannot be a possible hydrolysis product of I.

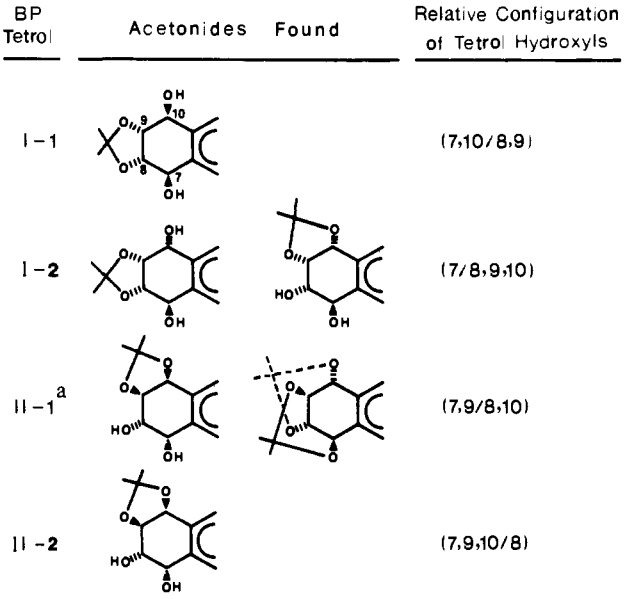


Figure 6. Structural identification of BP tetrols and their acetonide derivatives. Tetrols are those isolated from the hydrolysis of BP diol epoxides I and II (Figure 1). Acetonides are those resolved on HPLC (Figure 2). (a) The monoacetonide of tetrol II-1 is found to be a cis 9,10-acetonide formed from (7,9/8,10)-tetrol catalyzed by copper sulfate in anhydrous acetone.

Tetrol I-1 yields one monoacetonide and its mass spectrum is different from any of the tetrol acetonides derived from II (Fig. 3). The mass spectrum of the tetrol I-1 monoacetonide conforms well with an 8,9-monoacetonide derivative of either (7,10/8,9) or (7/8,9,10) configurations. The presence of *m/e* 260 ion is diagnostic of an 8,9-acetonide (Figure 5C), or rather of free hydroxyls at both C(7) and C(10) as it is formed through a reverse Diels–Alder cleavage of the terminal ring.[32] This fragmentation pathway is further supported by the observation that the $^{14}C(7)$ and also C(10)–^{18}O labeled acetonide derivatives exhibited the analogous ions at *m/e* 262 and at *m/e* 244. These data also support the mechanism that the *m/e* 260 ion is formed by cleavage of the C(7)–C(8) and C(9)–C(10) bonds. The formation of *m/e* 285 ion again can be explained by the concomitant loss of a methyl radical and acetic acid, while the *m/e* 284 ion can be represented as an ionized diphenol.

Tetrol I-2 yielded two acetonides in nearly a 1:1 ratio (Figure 2). The mass spectrum of the faster eluting acetonide of tetrol I-2 on HPLC (Figure 2) was found to be very similar to that of the 8,9-acetonide of tetrol I-1, exhibiting the significant ions mentioned above. However, a more intense ion at *m/e* 284 which represents an ionized 7,10-diphenol and a small ion at *m/e* 242 were found. The spectrum of the second acetonide of tetrol I-2 was in all respects identical with that of the 9,10-acetonide of tetrol II-1, but exhibited a relatively less intense *m/e* 285 ion and a significant *m/e* 284 ion. Apparently the propensity for electron impact induced combined loss of methyl radical and acetic acid is dependent on the stereochemistry of the hydroxyl groups. Thus the data presented above provide evidence that a tetrol with (7,10/8,9) configurations can only form one cis 1,2-monoacetonide which bridges carbons at 8 and 9 positions. Similarly a tetrol with (7/8,9,10) configurations can form two cis 1,2-monoacetonides bridging either the 8,9 or the 9,10 carbons. The results above thus establish that tetrols I-1 and I-2 are (7,10/8,9)-tetrol and (7/

8,9,10)-tetrol, respectively.

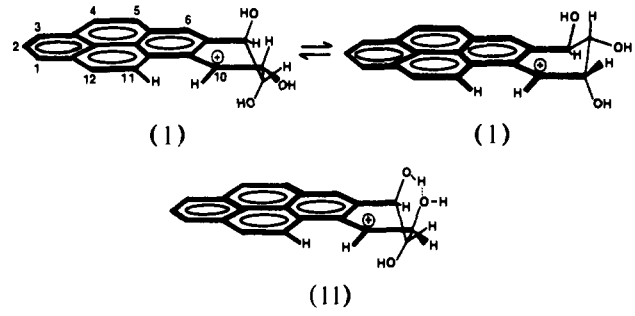
Considerations of the above data allowed us to make unambiguous structural assignments to the four stereoisomeric BP tetrols and their acetonides. The above assignments are summarized in Figure 6 and are consistent with results of the vicinal cis diol test (Table I).

We have established from the structure of the tetrols (Figure 6) and the tetrol ratios (Figure 1) that hydrolysis proceeds trans stereoselectively for I and cis stereoselectively for II. The structure of the tetrols indicates that the tetrols are formed by cleavage of C(10)–O bonds of the diol epoxides. If the initial epoxide ring opening had occurred at the C(9)–O bond, one would expect to find at least a minor amount of the trans addition product (7,9/8,10)-tetrol in addition to the cis addition product (7/8,9,10)-tetrol in the hydrolysis of I, and a minor amount of the trans addition product (7,10/8,9)-tetrol in addition to the cis addition product (7,9,10/8)-tetrol in the hydrolysis of II. Since (7,9/8,10)-tetrol and (7,10/8,9)-tetrol were not found as products in the hydrolysis of I and II, respectively, the epoxide ring opening at the C(9)–O bond does not occur.

The formation of two tetrols through the C(10)–O bond cleavage of I indicates that the mechanism of hydrolysis is an S_N1 reaction. This is further supported by a kinetic study which showed that the mechanism for the hydrolysis of I is a specific- and general-acid-catalyzed first-order reaction and the reaction intermediate is a carbonium ion.[33] A carbonium ion intermediate of I can exist in two conformations (Figure 7), and nucleophilic attack can occur from either side of the planar benzylic carbonium ion. Both conformations favor trans (to the C(9) OH) nucleophilic attack. This is consistent with the result that the (7,10/8,9)-tetrol is the major product found.

Diol epoxide II yields on hydrolysis a major (7,9,10/8)-tetrol and a minor (7,9/8,10)-tetrol. In contrast to diol epoxide I, the major tetrol from diol epoxide II is the cis (to the C(9) OH) addition product. This observation can best be explained by the conformation of a carbonium ion intermediate involving the hydrogen bonding between C(7) OH and C(9) OH (Figure 7). Since the carbonium ion is at C(10), the carbons at 9, 10, and 7 positions are on the same plane as the pyrene ring. Therefore the carbonium ion intermediate of diol epoxide II can exist in only one conformation in which the C(8) OH is below (or above) and slightly pointing to the center of the ring. The configuration of this C(8) OH would therefore exhibit steric hindrance toward the incoming nucleophiles from the side which is trans to C(9) OH. The hydrogen-bonded C(7) OH and C(9) OH, however, are pointing away from the center of the ring and its steric hindrance toward the incoming nucleophiles would be less than that of C(8) OH. Our results suggest, however, that the steric hindrance of the C(8) OH plays a major mechanistic role in the formation of the major cis addition product, (7,9,10/8)-tetrol. A carbonium ion intermediate without hydrogen bonding between C(7) OH and C(9) OH can exist in two conformations, and the trans (to the C(9) OH) addition product would be expected to be the major product as similarly depicted for that of diol epoxide I (Figure 7). Since the major hydrolysis product was found to be the cis addition product (7,9,10/8)-tetrol, we suggest that the carbonium ion intermediate in the hydrolysis of diol epoxide II is a hydrogen-bonded structure (Figure 7). The result that the (7,9,10/8)-tetrol is the major product in the acid-catalyzed exchange reaction of the (7,9/8,10)-tetrol at C(10) position supports the proposed hydrogen-bonded conformation of the carbonium ion intermediate.

Our results indicate that the carbonium ions derived from protonation of the 9,10-epoxide are the intermediates in the hydrolysis of both diol epoxides I and II. Thus both diol epoxides exhibit carbonium ion properties in the aqueous medium which could be important in their reactivity toward cellular nucleophiles.



(I) (I)

(II)

Figure 7. Proposed conformations of the carbonium ion intermediates of BP diol epoxide I and diol epoxide II. Thick lines indicate that they are on the same plane with the pyrene ring. Triangles indicate that they are above the plane of the paper.

BP diol epoxides I and II are very reactive molecules[11] and diol epoxide I has been postulated to be the predominant carcinogenic form of BP.[2,4,9,12,15,16,18,34] Hulbert[35] has postulated that diol epoxide II will react with nucleophiles by an S_N1 mechanism whereas diol epoxide I will react predominantly by an S_N2 mechanism. Yagi et al.[11] reported from their study of reaction rates in dry *tert*-butyl alcohol that diol epoxide II is 160-fold more reactive than diol epoxide I. Beland and Harvey[14] reported that reactions of diol epoxides I and II with the strong nucleophile *tert*-butyl thiolate in aqueous dioxane formed the respective products of trans specific ring opening. We have found that, in aqueous media, two tetrols were formed from each of the two stereoisomeric diol epoxides through carbonium ion intermediates at C(10).[36]

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References and Notes

(1) (a) Chemistry Branch, NCI. (b) C. M. T. Branch, NCI. (c) Abbreviations: BP, benzo[*a*]pyrene; HPLC, high-pressure liquid chromatography; (7,9/8,10)-tetrol, the C(9) OH is cis and the C(8) OH and C(10) OH are trans to the "reference" C(7) OH, respectively. See ref 12 for the nomenclature for BP diol epoxides I and II. All compounds described in this paper are racemic.
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The Mechanism of Hydrolysis of the Non-K-Region Benzo[*a*]pyrene Diol Epoxide *r*-7,*t*-8-Dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene

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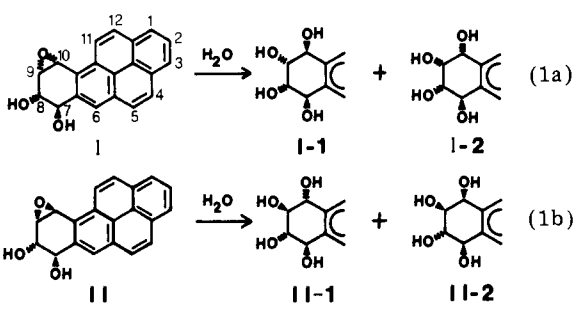
Abstract: The non-K-region benzo[*a*]pyrene diol epoxide *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, a potent mutagen and possibly the ultimate carcinogenic form of benzo[*a*]pyrene, has been found to undergo specific- and general-acid-catalyzed hydrolysis. The kinetics of hydrolysis was studied in 5% (v/v) aqueous tetrahydrofuran solutions at 25 °C with varying concentrations of buffer and ionic strength. The pH of the solutions was controlled by buffers of which the conjugated acid (HB) bears a negative ($H_2PO_4^-$), neutral (CH_3COOH), or positive ($(CH_2OH)_3CNH_3^+$) charge. The observed first-order rate constants (k_{obsd}) are linearly proportional to the buffer concentrations at constant pH. With increasing ionic strength at constant pH, k_{obsd} increases in Tris buffer, remains relatively constant in acetate buffer, and decreases in phosphate buffer. The observed rate constants can be expressed as $k_{obsd} = K_{eq,H}k_H[H^+] + K_{eq,HB}k_{HB}[HB]$. The value for $K_{eq,H}k_H$ is 1000 s^{-1} M^{-1} and for $K_{eq,HB}k_{HB}$ (s^{-1} M^{-1}) are 1.21, 1.03, and 0.07 for $H_2PO_4^-$, CH_3COOH, and $(CH_2OH)_3CNH_3^+$, respectively. These results indicate that the mechanism of hydrolysis involves a prior equilibrium (with equilibrium constant $K_{eq,H}$ and $K_{eq,HB}$) involving hydrogen bonding of the diol epoxide with an acid followed by a rate-determining proton transfer to form a benzylic carbonium ion intermediate at C(10). The planar carbonium ion intermediate undergoes an S_N1 nucleophilic attack by solvent water to form a (7,10/8,9)-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and a (7/8,9,10)-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in an approximately 3:1 ratio. The relative amounts of the two stereoisomeric tetrahydroxytetrahydrobenzo[*a*]pyrenes (tetrols) were analyzed by high-pressure liquid chromatography and the formation of the major tetrol was found to decrease slightly with increasing pH.

The diol epoxide *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (I) is more highly mutagenic in mammalian cells than 15 other benzo[*a*]pyrene (BP) derivatives including the stereoisomeric diol epoxide *r*-7,*t*-8-dihydroxy-*c*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (II).[1] A single enantiomer of I is formed predominantly from BP via the (−)-*r*-7,*t*-8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP (−)-*trans*-7,8-diol) by the mammalian microsomal mixed-function oxidases[2,3] and it is the major form bound to mammalian cellular DNA and RNA. I and II are each hydrolyzed in aqueous medium to a pair of stereoisomeric tetrahydroxytetrahydrobenzo[*a*]pyrenes (tetrols I-1, I-2, II-1, and II-2) and the stereochemistry of the tetrols has been elucidated.[3,7,8] The finding that I is formed predominantly in the biological system prompted us to carry out a detailed kinetic study of the hydrolysis of I. The results indicate that the hydrolysis is a specific- and general-acid-catalyzed S_N1 reaction and the intermediate is a benzylic carbonium ion at C(10). A carbonium ion intermediate indicates that diol epoxide I can react with cellular macromolecules as an alkylating agent.



(1a)

(1b)

Experimental Section

Materials. Synthetic diol epoxides I and [7-^{14}C]diol epoxide I were obtained through National Cancer Institute Contract NO1-CP-