Internal Hydrogen Bonding in Benzo[*a*]pyrene Diol and Diol Epoxide Metabolites

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Fluorescence, excitation, and emission spectra of some benzo[*a*]pyrene diols and diol epoxides in aqueous and non-aqueous media have been studied and ground- and excited-state dissociation constants have been determined. Spectral shifts due to changes in solvation, Stokes shifts, and pK_a and pK_a^* values are employed to show that the diols and diol epoxides possess an intramolecular hydrogen bond between *ortho* hydroxy groups when in non-aqueous media. In aqueous media no internal hydrogen bonding occurs either between ortho hydroxy groups or hydroxy and epoxide groups. The latter point is significant with regard to the carcinogenic reactivity of *anti*- and *syn*-7,8-dihydroxy 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

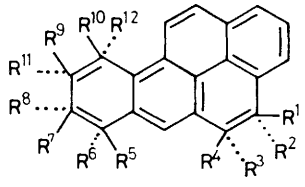
During mammalian metabolism a large portion of ingested polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene are transformed into dihydro diols. The latter are precursors of the mutagenic diol epoxides, the most active of which is *trans*-7,8-dihydroxy 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

Hulbert[1] predicted that the chemical reactivity of *syn* diol epoxides would be greater than that of the *anti* isomers toward nucleophiles owing to an internal hydrogen bond between a pseudoaxial β-hydroxy group and the epoxide oxygen. For *trans*-7,8-dihydroxy 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene, this is the 7-hydroxy group. A similar hydrogen bond is not present in the *anti* isomer owing to steric constraints. The *syn* isomer was shown to be more reactive toward nucleophiles than the *anti* isomer in pure organic solvent systems,[2–4] but they have almost equal reactivity in aqueous organic systems.[4] The data gathered in organic solvent systems appears to support the idea that an intramolecular hydrogen bond exists in *syn*-diol epoxides, however, data collected in aqueous organic solvent systems only support hydrogen bonding to the solvent. Another study[5] presents data and arguments which indicate that molecular conformation rather than internal hydrogen bonding is the important feature in the rate of solvolysis in water. Two additional studies present CNDO/2[6] and MNDO/3[7] theoretical calculations which found no evidence for internal hydroxy–epoxide hydrogen bonding in the *syn* isomer.[6,7] The most recent study supports the idea of an internal hydrogen bond in the crystalline state and in 80% water–dioxane for a similar naphthalene derivative which can assume a *syn* disposition.[8]

The present study was undertaken as a result of our long-term interest in the environmental effect of solvents, acids, and bases on the electronic spectra of polycyclic aromatic hydrocarbons. In addition it was thought that fluorescence spectroscopy would be useful in determining whether a hydroxy–epoxide hydrogen bond exists in the ultimate carcinogen *trans*-7,8-dihydroxy 9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

Experimental

Materials.—The benzo[*a*]pyrenes (BP), 4-hydroxy-BP (4-OH-BP), 7-hydroxy-BP (7-OH-BP), 4,5-dihydroxy-BP (4,5-DBP), 4,5-dihydro(epoxy)-BP (4,5-BPE), *trans*- and *cis*-7,8-dihydro-7,8-dihydroxy-BP (7,8-DBP), and both the *anti* and *syn* isomers of *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-BP (7,8-DBP-9,10-E) were supplied by J. N. Keith, ITT Research Institute, Chicago, Illionois in lots of 5 mg or less (Figure 1). All compounds were used without further purification but were determined to be pure by comparing the



	R											
	1	2	3	4	5	6	7	8	9	10	11	12
4,5-DBP, *cis*	H	OH	OH	H	H	H	H	H	H	H	H	H
trans	OH	OH	H	H	H	H	H	H	H	H	H	H
4,5-BPE	H	–O–		H	H	H	H	H	H	H	H	H
4-OH-BP	OH	—	—	—	H	H	H	H	H	H	H	H
7,8-DBP, *cis*	H	H	H	H	OH	H	OH	H	H	H	H	H
trans	H	H	H	H	H	OH	OH	H	H	H	H	H
7-OH-BP	H	H	H	H	OH	—	—	—	H	H	H	H
7,8-DBP-9,10-E, *anti*	H	H	H	H	H	OH	OH	H	–O–		H	H
syn	H	H	H	H	H	OH	OH	H	H	H	–O–	

Figure 1. Structures of the benzo[*a*]pyrenes studied.

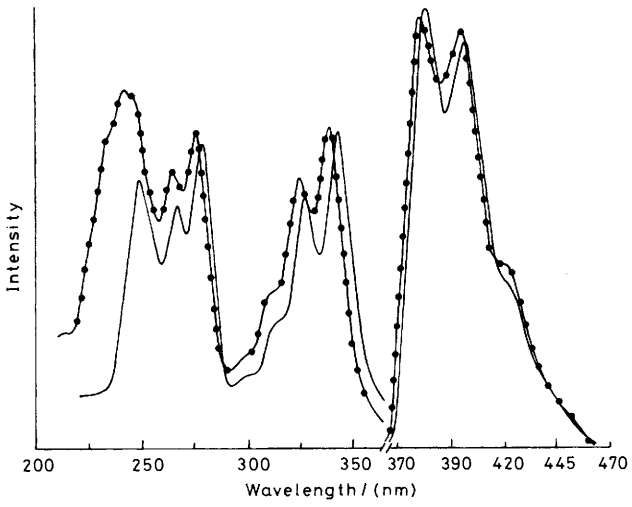


Figure 2. Spectra of *syn*-7,8-DBP-9,10-E in chloroform (——) and absolute methanol (— · — · —); intensity in arbitrary units.

respective absorption and corrected fluorescence excitation spectra of their dilute ethanolic solutions, and by their fluorescence emission spectra. The melting points provided by the supplier were verified. Stock ethanolic solutions (*ca.* 1.0×10^{-6} mol dm^{-3}) of the agents were prepared fresh daily, and stored in the dark at 10 °C. Solid samples were kept in a freezer at −10 °C. Basic solutions were prepared by dilution of carbonate-free sodium hydroxide with distilled, deionized water. The latter was also used to make acidic solutions from reagent grade sulphuric acid. Sulphuric acid solutions were calibrated by means of the corrected Hammett acidity scale of Jorgenson and Hartter.[9] Solutions for the determination of pK_a and pK_a* values were prepared by dilution of sulphuric acid or sodium hydroxide solution. Buffer solutions were not used because of their possible interference in fluorimetric titrimetry.[10] The solvents tetrahydrofuran and cyclohexane were dried over calcium hydride, distilled before use, and used for individual spectral measurements.

Methods.—Solutions for fluorimetric study of acid–base behaviour were prepared by adding the stock ethanolic hydrocarbon solution (100 mm^3) to the acid or base solution (2 cm^3) contained in a 2 cm^3 cuvette positioned in the spectrometer, using a volumetric pipette. The addition of the ethanolic hydrocarbon solution to the acidic or basic solutions resulted in a 95% aqueous alcoholic solution which had no apparent effect on the spectra. After delivery of the hydrocarbon, each solution was rapidly mixed and its maximum was recorded immediately. The total recording time for each point was no more than 2 s in order to minimize decomposition problems. All acid–base studies were conducted in triplicate. Values of pK_a and pK_a* were obtained graphically from respective plots of the normalized fluorescence excitation and emission intensity (I_f/I_{fo}) *vs.* Hammett acidity or pH. Triplicate fluoresence excitation and emission spectra were recorded for all agents in cyclohexane and chloroform after having dissolved a small quantity of the pure solid in the dried solvent. Triplicate spectra were recorded for all compounds except 4,5-BPE in aqueous buffer solution; 4-OH-BP and 7-OH-BP were also included in this experiment. Triplicate excitation and emission spectra were recorded for *anti*- and *syn*-7,8-DBP-9,10-E, in methanol. Blank spectra of all solvents were recorded at maximum bandpass and amplification to assure that no fluorescent impurities were present. The fluorescence excitation and emission spectra of *anti*- and *syn*-7,8-DBP-9,10-E (1×10^{-6} mol dm^{-3}) were also recorded, as a function of time in dry and aqueous tetrahydrofuran solution (95%), respectively, by dissolving a small quantity of the pure solid in dry tetrahydrofuran and then diluting it with water. All spectra of solutions containing water were recorded in 6 s increments, 12 nm long, to avoid the possibility of recording decomposition products. Therefore, a spectrum extending from 300 to 360 nm is composed of 5 separate increments from 5 different experiments.

Results

Table 1 shows that the fluorescence-excitation spectra for both *cis*-and *trans*-4,5-DBP lie at longer wavelength when these compounds are in cyclohexane or chloroform than when they are in buffer solution. Similar results are obtained for *cis*- and *trans*-7,8-DBP, and *anti*- and *syn*-7,8-DBP-9,10-E (Figure 2). The entire spectrum for each of the last two isomers did not shift to longer wavelengths (red shift) when the solvent was changed from buffer solution or methanol to the other non-polar solvents. Instead the solvent-induced spectral shift was observed only for spectral bands, 4, 3, and 2. Band 1 is broad, diffuse, and of low intensity, and no discernible solvent-induced shift was observed upon changing solvents even though one may be present. The spectrum of *syn*-7,8-DBP-9,10-E lies at longer wavelength than the *anti* isomer in buffer and methanol, but in the other solvents spectra of each isomer are essentially the same, and correspond with published values.[11] The results for 4,5-BPE were the opposite of those obtained for 4,5-DBP. The former's spectrum not only shifted to shorter wavelengths (blue shift) in cyclohexane and chloroform, relative to that obtained in buffer solution, but also dramatically changed shape to the extent that it resembled more a derivative of chrysene than of pyrene when in non-aqueous solution (Table 1). Its spectrum in buffer solution was identical with that of 4-OH-BP.

Although the emission spectra of the above compounds also demonstrate solvent dependence, they did not, however, always parallel the dependences observed in their excitation spectra (Table 1). The emission spectra of 4,5-DBP for both the *trans* and *cis* isomer undergo a red shift upon changing the solvent from buffer solution to cyclohexane or chloroform. Different results were obtained for the *trans* and *cis* isomers of 7,8-DBP; the spectrum of the *cis* form demonstrated a greater red shift than did the *trans* form when the solvents were changed as described above. However, both the *syn* and the *anti* isomers of 7,8-DBP-9,10-E demonstrated a red shift upon the changing solvents. Furthermore, the spectrum of the *syn* isomer in buffer solution or methanol has its band maxima at slightly longer wavelengths than that of the *anti* isomer; in cyclohexane and chloroform the maxima of each isomer are the same.

The emission spectrum of 4,5-BPE in cyclohexane and chloroform compared with that in buffer solution resembles the corresponding excitation spectrum in that a blue shift is observed and it has a chrysene-like appearance, and its spectrum recorded in buffer solution is identical with that of 4-OH-BP.

The fluorescence emission from *anti*- and *syn*-7,8-DBP-9,10-E in dry tetrahydrofuran is constant and stable for at least two days in the dark, however, under normal laboratory lighting their emission intensity increases with time. In each case the spectra obtained resembled those recorded in cyclohexane or chloroform with regard to their wavelength position. In aqueous tetrahydrofuran (95% dry tetrahydrofuran and 5% water), the emission intensity is enhanced with time in both the dark and the light. The spectra obtained in aqueous tetrahydrofuran lies to the blue of those obtained in dry tetrahydrofuran

Other workers have reported similar results in regard to

Table 1. The corrected fluorescence-excitation and emission spectral features and Stokes shifts (SS) of the benzo[*a*]pyrenes examined, in buffer solution (B), chloroform (H), and cyclohexane (C).

Compound		Excitation/nm									SS	Emission/nm		
		4[a]		3[a]		2[a]				1[a]	10^{-4} cm^{-1}			
4,5-DBP, *trans*	B			(262)[b]	273[f]	297[f]	309	323	349	364	0.37	366[f]	385	406
	C,H			(264)[b]	275[f]	299[f]	312	326	352	367	0.36	369[f]	388	409
4,5-DBP, *cis*	B			(262)[b]	273[f]	297	309	323	349	364	0.37	366	385	406
	C, H			(264)[b]	275[f]	299[f]	312	326	352	367	0.36	369	388	409
4,5-BPE (4-OH-BP)	B	(257)[b]	264[f]	288	298[f]	353	373[f]	388	409	—	—	425[f]	445	—
4,5-BPE	C, H	—	—	262	273[f]	297[f]	309	323	364	—	—	369[f]	389	411
7,8-DBP, *trans*	B	(230)[b]	257[f]	281	292[f]	333	349	366[f]	396	—	0.24	402[f]	424	446
	C, H	—	258	284	294[f]	337	351	369[f]	396	—	0.22	403	425	447
7,8-DBP, *cis*	B	(230)[b]	257[f]	281	292[f]	333	349	366[f]	396	—	0.24	401[f]	422	444
	C, H	—	258[f]	284	294[f]	337	351	269[f]	396	—	0.22	402[f]	424	447
7,8-DBP-9,10-E, *anti*	B, A,[c] M[d]	(235)[b]	245[f]	266	277[f]	313	327	342[f]	367	378	0.29	380[f]	399	420
	T, C, H	(239)[b]	248[f]	269	281[f]	318	331	347[f]	367	378	0.28	384[f]	402	424
7,8-DBP-9,10-E, *syn*	B, A,[c] M[d]	(236)[b]	246[f]	267	278[f]	314	328	344[f]	367	378	0.29	381[f]	400	421
	T,[e] C, H	(238)[b]	248[f]	269	281[f]	318	331	347[f]	367	378	0.28	384[f]	402	424
4-OH-BP	B	257	264[f]	287	297[f]	353	373	388	409	—	—	425	445	—
7-OH-BP	B	—	265	290	300[f]	355	376[f]	394	(415)	—	—	—	460	—

[a] Spectral band assignments have not been made for these compounds, except 4-OH-BP and 7-OH-BP (ref. 27); in the latter two phenols, bands 1–4 have the same assignments as pyrene, ^1L_b, ^1L_a, ^1B_b, and ^1B_a, respectively. [b] Values in parentheses are estimated from vibrational shoulders. [c] A = Aqueous tetrahydrofuran (1:1). [d] M = Absolute method. [e] T = Tetrahydrofuran. [f] Band maxima.

Table 2. Ground-(pK_a) and lowest-excited singlet state (pK_a*) ionization constants for the BP diols and diol epoxides in this study.

BP	pK_a	Wavelength/nm	pK_a*	Wavelength/nm
4,5-DBP, *trans*	12.7[a]	325	−1[a]	385
4,5-DBP, *cis*	13.5	325	−0	385
4,5-BPE (4-OH-BP)	9.4	260	1.7	425, 550
7,8-DBP, *trans*	11.8[a]	368	1.0[a]	403
7,8-DPB, *cis*	11.9	368	1.0	403
7,8-DBP-9,10-E, *anti*	12.0	343	1.0	405
7,8-DBP-9,10-E, *syn*	12.1	343	1.0	405

[a] Ref. 18.

emission intensity enhancement.[12,13] Our results conflict with theirs in that we observed *anti*-7,8-DBP-9,10-E fluorescence, not only in tetrahydrofuran, but in buffer solution, cyclohexane, and chloroform as well. In order to resolve the conflict, care was taken to record the spectra of *anti*- and *syn*-7,8-DBP-9,10-E so that only these isomers, and not their primary hydrolysis product 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydro-BP were recorded as described in the experimental section. In addition, the course of the hydrolysis was followed fluorimetrically in aqueous solution. The fluorescence bands of the product overlaid those of the isomers but the former displayed much greater intensity. The relative fluorescence intensity for the product was found to be ten times greater than that for the *anti* isomer and 20 times greater than the *syn* isomer. Semilogarithmic plots of the normalized fluorescence intensity *versus* time yielded curvilinear plots which reached a plateau at *ca.* 10 min. Rate constants calculated from the initial linear portions were 2.6×10^{-3} s^{-1} and 1.1×10^{-3} s^{-1}, respectively, for the *syn* and *anti* isomers, statistically different, and similar in magnitude to those reported elsewhere.[11,13] The above plots were similar to those reported elsewhere.[13]

The excitation and emission spectral intensities for all compounds except 4,5-BPE are constant in the neutral pH region but were quenched as either the basicity or the acidity was increased. The mid-points at which this occurred are listed (Table 2). At the end-point of the acid–base reaction no new spectral bands were observed, and when the amplifier sensitivity were increased the vibrational fine-structure characteristics of each compound at neutral pH were retained. For *cis*- and *trans*-4,5-DBP the quenching of the emission spectra ceases at H_o −4.7 when the spectral band suddenly loses all vibrational features and presents as a diffuse band overlying the original band; we attribute this to decomposition. The midpoint of quenching was estimated (Table 2). For 4,5-BPE, a partial quenching of the entire spectral band at neutral pH was observed and exactly accompanied by the appearance of a new spectral band at longer wavelength which partially overlapped the original band. Both bands looked exactly like the neutral and anionic species, respectively, for 4-OH-BP, and did not resemble these species for 5-OH-BP. The emission spectrum of 4,5-BPE behaved as its excitation spectrum, except that the band observed at neutral pH was completely quenched as a new band appeared at longer wavelength with increasing solution acidity.

The pK_a values for *cis*- and *trans*-4,5-DBP differ by nearly one log unit, and each are greater than the values we report for 7,8-DBP and 7,8-DBP-9,10-E (Table 2). However, the values for *cis*- and *trans*-7,8-DBP and *syn*- and *anti*-7,8-DBP-9,10-E are the same not only for each isomer but each compound. In the lowest excited singlet state the acid dissociation constants are identical for the stereoisomers of 7,8-DBP and 7,8-DBP-9,10-E, and for 4,5-DBP they differ by one log unit. The pK_a value for 4,5-BPE is identical with that reported for 4-OH-BP.

Discussion

The red shift in the excitation and emission spectra of all compounds except 4,5-BPE when the solvent is changed from buffer solution to either cyclohexane or chloroform is anomalous for compounds that are capable of undergoing only π–π* transitions (Table 1).[14–16] Usually these types of compounds have their electronic spectra blue-shifted as solvent polarity and hydrogen-bonding capability are decreased, as exemplified by BP, 3-OH-BP and 9-OH-BP.[17] The result that the compounds examined exhibit opposite behaviour indicates that a solvent-related physical interaction is responsible for the red shift. The compounds that demonstrate the shift have *ortho*-

disposed hydroxy groups and it is possible that these groups are hydrogen bonded to water when in aqueous media. In organic media competition with water for the hydroxy groups is non-existent, and conditions are optimal for the two groups to form an intramolecular hydrogen bond. This can be seen by comparing the spectral shifts for 7,8-DBP-9,10-E in water-free and aqueous tetrahydrofuran (Table 1). The addition of water to dry tetrahydrofuran blue-shifts both excitation and emission spectra by reducing the intramolecular charge transfer to the aromatic ring. In these compounds this could only be due to decreased electron delocalization caused by disrupting the five-membered ring created during intramolecular hydrogen bonding of the hydroxy groups. Similar conditions probably exist in cyclohexane and chloroform.

The similarities between 7,8-DBP and 7,8-DBP-9,10-E with regard to not only their solvent-induced spectral shifts but their pK_a and pK_a* values are noteworthy. The latter are the same for the *cis* and *trans* isomers and the *anti* and *syn* isomers of the above agents, respectively (Table 2). This indicates that the deprotonation site is the same for each molecule and is most likely the 7-OH group. This appears to be the case since in each molecule (or isomer) the 7-position is benzylic whereas the 8-position is either allylic or alkyl-like for 7,8-DBP and 7,8-DBP-9,10-E, respectively. If the 8-OH group were the one undergoing protolysis a difference in pK_a value would be expected between these two molecules owing to the electronic charge differences in the two substitution sites.

The importance of this point and the previous one regarding the hydrogen-bonded hydroxy groups concerns conflicting literature reports on the existence of an internal hydrogen bond in the *syn* isomer. The results we report in Tables 1 and 2 strongly indicate that hydroxy–epoxide hydrogen bonding does not occur in the *syn* isomer in aqueous or non-aqueous media for the following reasons. (*a*) The fluorescence-excitation and emission spectra of the *syn* isomer are positioned at longer wavelength than those of the *anti* isomer. This indicates an increase in intramolecular charge transfer by the hydroxy groups(s) on the *syn* isomer relative to the *anti* isomer. This point constitutes very strong primary evidence that the *syn* isomer assumes a more planar conformation than that assumed by either the *anti* isomer or especially the conformation that hydroxy–epoxide hydrogen bonding would dictate. Evidently each isomer exists primarily with the 7- and 8-hydroxy groups in a quasi-diequatorial position when in aqueous solution such that an internal hydrogen bond cannot form. (*b*) Both the *syn* and *anti* isomer have the same pK_a and pK_a* values indicates that the site of deprotonation, and its immediately adjacent areas on each isomer have the same relative conformational freedom. This would not be true if one isomer was engaged in hydrogen bonding while the other was not. (*c*) The identical Stokes shift for the *syn* and *anti* isomers in each solvent, but particularly in buffer solution shows that they undergo the same solvent relaxation processes as their optical electrons pass from the Franck–Condon excited state to the lowest excited singlet state. This means that the two isomers have very similar ground- and excited-state solvent configurations and dipole moments. This point is supported by the result that the fluorescence-excitation and emission spectra of the two isomers are identical with regard to band shape and vibrational features.

The more basic nature of *cis*-4,5-DBP in both the ground- and lowest-excited-singlet state, when compared with the *trans* isomer, may be due to the difference in planarity of the two isomers. This point becomes clearer after considering that the pK_a values for both isomers of 4,5-DBP are greater than those for either 7,8-DBP or 7,8-DBP-9,10-E, but about the same as that reported for 9,10-DBP.[18] The 9- and 10-position in the latter agent have been shown to be diaxial, in contrast with the diequatorial 7- and 8-positions.[8,19,20] The relatively greater ground-state basicities for *cis*- and *trans*-4,5-DBP may indicate that the 4- and 5-position are also diaxial. However, the 4- and 5-hydroxy groups in *trans*-4,5-DBP must be more in-plane with the aromatic ring since it is more acidic than the *cis* isomer in both ground- and lowest-excited-singlet states.

In buffer solution, 4,5-BPE appears to undergo solvolysis to 4-OH-BP since its excitation and emission spectra are identical with those of the latter compound and do not resemble those of 5-OH-BP. Formation of the phenol apparently from the oxide occurs by way of a [1,2]-hydride shift (NIH shift)[21] similar to that described for benzene and naphthalene oxide, and the oxides of phenanthrene and 3-methylcholanthracene.[22–24] This conclusion is supported by the pK_a and pK_a* values determined for 4,5-BPE which are the same as those reported[25] for the above phenol (Table 2).

The current biological significance of our results concerns the mutagenic, carcinogenic, and tumourigenic potencies of *anti*- and *syn*-7,8-DBP-9,10-E.[26–34] In each case the *anti* isomer is more active in mammalian systems than the *syn* isomer, and binds to DNA to a greater extent.[35,36] The *syn* isomer is chemically more active toward nucleophiles than the *anti* isomer.[2–4] Our results show that a hydroxy–hydroxy intramolecular hydrogen bond exists in each isomer only in non-polar media and does not involve the epoxide moiety. In aqueous media the 7- and 8-hydroxy groups are probably hydrogen-bonded to the solvent and probably exist in a quasi-diequatorial position in each isomer. The difference in biological and chemical activity between the two isomers may arise from differences in their relative conformations. In particular, molecular planarity rather than the relative reactivity of the epoxide moiety as controlled by an internal hydrogen bond may dictate their activity.

Acknowledgements

This work was supported, in part, by the National Cancer Institute, Grant Number CA 20695, and The University of Georgia College of Pharmacy.

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Received 22*nd July* 1988; *Paper* 8/01206G