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A Study of Steric and Electronic Factors Governing the Position of Biofunctionalization of the Benz(a)anthracene Nucleus: Metabolism *in Vitro* of Fluoro- and Methyl-Substituted Analogs

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Metabolism of selected fluoro and methyl analogs of benz(a)anthracene were studied in induced and uninduced rat liver S-10 fractions. Examination of the various metabolites produced from these substrates led to the conclusion that both steric and electronic factors determine the preferential sites of bioactivation. A hypothesis is proposed to explain the results.

Previously we described the biotransformation of 7, 12-dimethylbenz(a)anthracene (DMBA) in various induced and uninduced S-10 fractions in the presence and absence of hepatic mixed function oxidase inhibitors (*1, 2*). The data obtained led us to conclude that various dihydrodiols and 7- and 12-hydroxymethyl metabolites were formed by the action of different enzyme complexes, a conclusion which reinforced the multiplicity of P450s relative to their structural (*3, 4*) and functional differences (*5–8*).

Studies employing various monofluro-DMBA derivatives have provided evidence that substitution of F for H on this hydrocarbon blocks metabolism at the position of substitution (*9, 10*), a result in agreement with a rationale originally proposed by Newman *et al.* (*11, 12*). In cultured Syrian hamster embryo cells, a cell system transformable by DMBA and other polycyclic aromatic hydrocarbons (PAH), a positive correlation of absolute binding of ^{3}H or ^{14}C radiolabel to the DNA with carcinogenicity was obtained using highly carcinogenic DMBA and 11F-DMBA, weakly carcinogenic 5F-DMBA, and noncarcinogenic 2F-DMBA analogs (*9, 10, 13*).

In this article we describe the comparative metabolism of the 2,3,4,5, and 6F-DMBA analogs, 6F-benz(a)anthracene (6F-BA), 7-methyl-BA(7MBA), 3F-7MBA, 5F-7MBA, 12MBA, and 6F-12MBA in phenobarbital-, β-naphthoflavone-, and 3-methylcholanthrene (3MC)-induced male Sprague–Dawley rat liver S-10 fractions. Based upon these studies we propose a hypothesis which serves to explain the preferential sites of biofunctionalization of these compounds relative to the parent PAH.

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EXPERIMENTAL PROCEDURES

Materials

Hydrocarbons: DMBA was purchased from Eastman Chemical Company and was checked for purity by high-pressure liquid chromatography (HPLC) prior to use and when necessary purified by column chromatography over silica gel (Silica Gel-60; E. Merck) using hexane : benzene (1 : 1) as eluant. The 2 and 3F-DMBA analogs were prepared as previously reported (*14*). 5F-DMBA was prepared from 1F-naphthalene via Friedel–Crafts acylation in CS_2 and subsequent known transformation of the resulting keto-carboxylic acid (*15*). 4F-DMBA, 7MBA, 12MBA, 3F-7MBA, and 5F-7MBA were gifts of Professor M. S. Newman of The Ohio State University. 6F-DMBA, 6F-BA, and 6F-12MBA were gifts of Professor Wolfgang Girke, Institute for Organische Chemie der Universitat, Frankfurt M. (*16*).

Methods

Generation of metabolites: Induction schedule I. Liver S-10 fractions were obtained from pretreated male Sprague–Dawley rats weighing 150–200 g (ip dose of phenobarbital Na salt was 100 mg/kg/day for 4 days; 3MC was 20 mg/kg/day for 2 days; β-napthoflavone was 75 mg/kg/day for 5 days).

Induction schedule II. Other batches of rat liver S-10 preparations were obtained from male Sprague–Dawley rats, 150–200 g (ip dose phenobarbital Na salt was 100 mg/kg/day for 4 days; 3MC was 20 mg/kg/day for 4 days; β-naphthoflavone was 75 mg/kg/day for 5 days).

Metabolites of PAH were generated as previously described (*2, 17–19*). For these studies all PAH were concurrently incubated for the same length of time with a S-10 fraction obtained from a single induction schedule. A blank and reference DMBA incubation always were included. We estimate our detection limit to be approximately 1% of total metabolites based upon utilization of radiolabeled DMBA and 2F-DMBA. Storage of S-10 fractions was as previously described (*2*), but in most cases they were used within one month of their preparation. All incubations contained considerable excess of PAH and for DMBA and 2F-DMBA double acetone–EtOAc extractions of the incubation medium furnished >95% of the activity in the organic phase. No tetrols were observed in this phase.

Isolation and characterization of metabolites. Isolation and characterization of metabolites was generally carried out under yellow light. Isolation by HPLC was carried out as previously described (*1, 2*). For spectral characterization of F analog metabolites we relied upon their characteristic uv spectra which are related to published spectra of BA analogs (*20–24*). Although the ultraviolet spectra for the various metabolites (dihydrodiols and phenols) and the order of elution of various fluoro compounds using water–methanol HPLC chromatography are the same as for DMBA metabolites, structures should be construed as tentative until synthetic standards are available. DHDs which were difficult to resolve by reverse phase Partisil HPLC were separated from the crude mixture of metabolites by thin-layer chromatography and chromatographed using HPLC over Laboratory

Data Control (LDC) Spherosorb Silica. This methodology insured the homogeneity of the individual peak and served as a source of material for further spectral characterization, but TLC was never empoyed as a prerequisit to quantification. All quantifications were carried out on the incubation mixtures.

Thus, to ensure the complete resolution of DHDs the ethyl acetate extract was spotted on silica gel thin-layer plates. The plates were developed in benzene: hexane (1 : 1) and the fluorescent band at $R_f = 0.82$ was scraped off. This material corresponded to unmetabolized PAH. Subsequently the plates were developed in benzene : ethyl acetate (7 : 3), visualized under uv light and divided into two fractions. Fraction A ($R_f = 0.7–1.0$) corresponded to phenols and monohydroxymethyl metabolites. Fraction B ($R_f = 0.1–0.6$) corresponded to dihydrodiols and perhaps small amounts of hydroxymethyl phenols. After elution of each fraction with MeOH or MeOH : EtOAc (1 : 1), the eluates were subjected to HPLC over LDC Spherosorb Silica 5 μ (4.6 mm × 25 cm), using a guard column (4.6 mm × 7 cm; packed with Lichoprep ™RP-18, 25–45 μm). The column was eluted with methanol in dichloromethane (0%, v/v, to 5%, v/v, or 0%, v/v, to 10%, v/v, in case of 7MBA metabolites) at a solvent flow of 2.0 ml/min over period of 60 min (linear gradient).

We examined the metabolism of DMBA and 2F-DMBA in phenobarbital and β-naphthoflavone S-10 fractions using both tritiated and cold material. Metabolites produced were quantified using an HPLC uv detector at 254, 260, and 270 nm and by scintillation counting. Radioactive quantification compared best with area measurements when the uv detector was at 270 nm. For uv peak areas $\geq$30% of total metabolites the correlation between radioactive quantification and area measurement was 2%, whereas peaks $\geq$10% and $\leq$29% were within 5% of area measurement and those $\geq$2% and $\leq$9% were within 10% of the area measurement. Since tritiated analogs for other PAH were not available, only relative amounts of metabolites formed could be assessed. However, based on the presumed similarity of extinction coefficients (*1, 2, 10, 20–22*) of F analog metabolites with the metabolite of the parent hydrocarbon the amount of total metabolites formed represent a good approximation. Contributions of phenols, including the corresponding 7- and 12-methyl hydroxylated compounds were consistently <6% of total metabolites (excluding phenol metabolites of 6F-BA and 6F-12MBA; Tables 4 and 5) and generally represented three or more components. Since these phenols were not rigidly characterized, they were not included in the sum of total metabolites representing functionalization at a given site on the PAH. Therefore, variations in DHD production <6% were not utilized in this semiquantitative analysis of PAH site-specific biofunctionalization. Furthermore, duplicate comparisons of functionalization at a given position were made using the same inducer, induction schedule, and dose and were within 2–3% of each other.

RESULTS

Production of 7- and 12-Methyl Hydroxylated Metabolites

Metabolism of all F-DMBA analogs in phenobarbital-induced S-10 fractions invariably furnished monohydroxy alcohols less polar than the corresponding

TABLE 1

RELATIVE RETENTION TIMES (min) OF METABOLITES OBTAINED FROM FLUORO AND METHYL ANALOGS ON WHATMAN PARTISIL PXS 10/25 ODS COLUMN

	DMBA analogs						BA analogs				
Metabolite	DMBA	2F	3F	4F	5F	6F	7M	3F-7M	5F-7M	12-M	6F-12M
7OHM	50.6	50.3	50.6	50.9	51.5	50.9	49.0	49.4	48.5	—[a]	—
12OHM	51.5	50.9	51.5	53.7	53.8	53.8	—	—	—	49.0	52.3
7,12-Bis-OHM	ND[b]	ND	40.6	42.0	41.9	43.8	—	—	—	—	—
3,4-DHD	47.4	ND	ND	ND	ND	47.8	47.0	ND	ND	46.3	46.8
7OHM-3,4-DHD	35.8	ND	ND	ND	ND	37.1	ND	ND	ND	—	—
5,6-DHD	34.8	36.3	34.4	33.1	ND	ND	31.9	32.5	ND	44.0	ND
7OHM-5,6-DHD	29.1	ND	ND	28.1	ND	ND	ND	ND	ND	—	—
8,9-DHD	37.8	38.3	38.4	40.0	40.0	40.0	35.6	36.5	38.5	44.4	46.0
7OHM-8,9-DHD	31.2	31.2	33.1	33.8	33.8	32.2	ND	30.5	ND	—	—
10,11-DHD	38.8	ND	ND	ND	40.6	40.3	33.0	33.8	35.6	35.0	39.0
7OHM-10,11-DHD	28.1	29.4	29.7	30.9	31.3	29.0	29.0	30.0	ND	—	—

[a] —Not detected or expected.
[b] Not detected.

7OHM analogs (Table 1). Since the uv (Table 2) and fluorescence spectra for these metabolites were identical to those of 7OHM, 12MBA, we assigned the 12OHM, 7MBA structure to the fluoro analogs. No detectable amounts of 12OHM metabolites were produced in β-napthoflavone- and 3MC-induced S-10 fractions. 7MBA and its 3F and 5F analogs also furnished the corresponding 7M-hydroxylated metabolites in both induced and uninduced S-10 fraction (Table 3). Conversely 12MBA and 6F-12MBA furnished the corresponding 12OHMBA and 6F-12OHMBA only in phenobarbital-induced S-10 fraction (Tables 1 and 3).

These results are consistent with our earlier hypothesis (*2*) that in DMBA, 7M- and 12M-hydroxylations proceed predominantly by independent enzyme systems. Using both induction schedules I and II, metabolism of DMBA and its 2, 5, and 6F analogs in phenobarbital-induced S-10 fractions furnished virtually equal amounts of 7OHM and 12OHM metabolites. However, 3F-DMBA yielded approximately 50% more 12OHM than 7OHM metabolites, and 4F-DMBA furnished approximately 33% more 7OHM than 12OHM compound. Additionally, the corresponding 7,12-bishydroxymethylbenzanthracenes were produced in relatively small amounts (<5%) from 3- and 5F-DMBA in phenobarbital-induced S-10 fractions (Table 2). Introduction of F at positions 4, 5, and 6 in DMBA generally caused a marked reduction in 7M-hydroxylation in both β-naphthoflavone- and 3MC-induced S-10 fractions (Table 3). Since these studies were carried out in the presence of excess substrate, results cannot be interpreted only on the basis of increased ring oxidation at the expense of methyl hydroxylation. Rather, it would appear that insertion of F not only blocks metabolism at the site of halogen substitution, but either owing to electronic or steric effects (*25, 26*) also inhibits metabolism of the methyl functions.

Production of 3,4-Dihydrodiols. Of all DMBA analogs examined only DMBA (*2*) and 6F-DMBA (Tables 1 and 2) yielded detectable amounts of the corresponding 3,4-DHDs in phenobarbital-induced S-10 fractions. In addition, 6F-BA and 6F-

TABLE 2

UV MAXIMA OF METABOLITES DERIVED FROM FLUORO DMBA ANALOGS

Compounds	uv Maxima(nm)
6F-DMBA-*trans*-3,4-DHD	273, 387(w), 407(w), 432(w)
6F-7OHM,12MBA-*trans*-3,4-DHD	271, 380–440(w)
2F-DMBA-*trans*-5,6-DHD	320–280(b), 268, 258
3F-DMBA-*trans*-5,6-DHD	320–280(b), 267, 258.5
4F-DMBA-*trans*-5,6-DHD	320–280(b), 267.5, 259
4F-7OHM,12MBA-*trans*-5,6-DHD	320–280(b), 266, 259
3F-DMBA-*trans*-8,9-DHD	330.5, 316, 284, 268
6F-DMBA-*trans*-10,11-DHD	308, 295(sh), 266
2F-7OHM,12MBA-*trans*-10,11-DHD	304(sh), 290(sh), 276
3F-7OHM,12MBA-*trans*-10,11-DHD	305, 291(sh), 277
4F-7OHM,12MBA-*trans*-10,11-DHD	308, 292, 275
5F-7OHM,12MBA-*trans*-10,11-DHD	306,288(sh), 278
6F-7OHM,12MBA-*trans*-10,11-DHD	309, 294(sh), 281
2F-7OHM,12MBA-*trans*-8,9-DHD	328, 313, 269
3F-7OHM,12MBA-*trans*-8,9-DHD	330, 316, 280(sh), 267
4F-7OHM,12MBA-*trans*-8,9-DHD	332.5, 318, 306(sh), 282(sh), 267
5F-7OHM,12MBA-*trans*-8,9-DHD	328, 316, 281, 268
6F-7OHM,12MBA-*trans*-8,9-DHD	332, 319, 283(sh), 267.5
2F-12OHM,7MBA	294, 283, 272, 263
3F-12OHM,7MBA	304(sh), 294.5, 284, 273, 263, 238
4F-12OHM,7MBA	305(sh), 296, 285.5(sh), 276, 263
5F-12OHM,7MBA	295.4, 284.7, 275(sh), 263
6F-12OHM,7MBA	305(sh), 293, 284.5, 275, 263
3F-7,12-Bis-OHM-BA	302(sh), 292.5, 282, 271, 261
5F-7,12-Bis-OHM-BA	295.5, 284, 273, 264

12MBA furnished substantial amounts of the corresponding 4-phenols in β-napthoflavone-induced (6F-BA 32 vs 6F-12MBA 16%) and 3MC-induced (6F-BA 31 vs 6F-12MBA 19%) S-10 fractions (Tables 4 and 5). Introduction of a methyl group at position 12 of BA markedly enhanced the production of the 3,4-DHD metabolite when compared to BA and 7MBA [induction schedule II: in the phenobarbital-induced S-10 fraction, BA = 3% (*23*), 7MBA = 3%, 12MBA = 14% (Table 1)]. The influence of 6F substitution on 3,4-DHD production is readily explained on electronic grounds (see below). The influence of the 12-methyl group is less easily explained but may reflect strain due to noncoplanarity of the molecule (*25*).

Production of 5,6-Dihydrodiols. Introduction of F in DMBA at position 4 substantially decreases production of 5,6-DHDs (induction schedule I: in 3MC-induced S-10 fraction, 3F-DMBA = 14%, 4F-DMBA = 8%) or completely eliminates their formation (induction schedule II: in 3MC-induced S-10 fraction, 3F-DMBA = 6%, 4F-DMBA = 1%; induction schedules I and II: in β-napthoflavone-induced S-10 fraction, 3F-DMBA = 6%, 4F-DMBA = not detected). In contrast to BA metabolism, which is reported to yield large quantities of 5,6-DHD (*20, 23*), introduction of a 7-methyl group afforded BA analogs (7MBA, DMBA) where 5,6-

TABLE 3

PRODUCTION OF 7- AND 12-METHYL HYDROXYLATED METABOLITES FROM METHYL AND FLUORO BA ANALOGS IN INDUCED MALE SPRAGUE–DAWLEY RAT LIVER S-10 FUNCTIONS

	Percentage of total metabolites			
	Phenobarbital-induced S-10		3-MC-induced S-10,	β-Napthoflavone-induced S-10,
Compounds	7OHM	12OHM	7OHM	7OHM
DMBA	18	18	20	18
2F-DMBA	19	17	14	24
3F-DMBA	19	29	17	19
4F-DMBA	33	25	2	3
5F-DMBA	26	30	8	6
6F-DMBA	33[a]	35[a]	8[a]	16[a]
7MBA	38	—[b]	17	20
3F-7MBA	53	—	29	33
5F-7MBA	59	—	16	8
12MBA	—	29	—	—
6F-12MBA	—	64	—	—

[a] Induction schedule I. All others are induction schedule II.
[b] —Not detected or expected.

DHD formation was reduced (Fig. 1). Similar to 3,4-DHD formation, introduction of a 12-methyl group had a stimulatory effect on 5,6-DHD yield (Tables 4 and 5). Influence of the 7-methyl group on 5,6-DHD production was similar in all induced fractions. Thus, biofunctionalization of the 5 and 6 positions is highly sensitive to substitution at neighboring position 4 and relatively less sensitive to substitution at position 7.

Production of 8,9-Dihydrodiols. Introduction of F at positions 4 or 5 of DMBA markedly enhanced production of 8,9-DHDs (Fig. 2, Tables 4 and 5) and inhibited biotransformation of rings A and B in β-napthoflavone- and 3MC-induced S-10 fraction. In the β-napthoflavone S-10 fraction either 7- or 12-methyl functions in BA provided a moderate decrease in 8,9-DHD formation (Tables 4 and 5). The presence of a 6F group in 12MBA and DMBA did not significantly affect the production of these DHDs in β-napthoflavone- and 3MC-induced S-10 fractions (Tables 4 and 5). However, introduction of F at position 3 of BA considerably inhibited the functionalization of the 8,9 double bond [induction schedule II: in β-napthoflavone-induced S-10 fraction, 8,9-DHDs in DMBA vs 3F-DMBA (50 vs 38%); in 7MBA vs 3F-7MBA (50 vs 32%)]. Unlike 5,6-DHD formation, 8,9-DHD yields seem to be relatively less sensitive to steric constraints, but are markedly influenced by electronic effects (see below).

Production of 10,11-Dihydrodiols. Of all DMBA analogs studied only the 3 and 6F compounds yielded relatively larger quantities of total 10,11-DHDs in β-naphthoflavone- and 3MC-induced S-10 fractions (Tables 4 and 5, Figs. 3A and 3B). Similarly, 3F-7MBA yielded 21–25% of 10,11-DHD, whereas 7MBA af-

TABLE 4

RELATIVE PERCENTAGE OF VARIOUS METABOLITES FROM FLUORO AND METHYL ANALOGS OF BA IN β-NAPTHOFLAVONE-INDUCED S-10 FRACTIONS

	DMBA analogs						BA analogs						
Metabolite	DMBA	2F	3F	4F	5F	6F	BA	6F	7M	3F-7M	5F-7M	12M	6F-12M
5,6-DHD	9	3.4	6	ND[a]	ND	ND	18	ND	5	1	ND	19	ND
7OHM,5,6-DHD	<1	ND	ND	ND	ND	ND	<1	ND	<1	<1	ND	<1	ND
8,9-DHD	47	45	29	62	68	37	70	44	48	25	49	44	45
7OHM-8,9-DHD	3	10	9	16	2	7	—[b]	2	2	7	1	ND	ND
10,11-DHD	<1	<1	ND	ND	ND	15	<1	<1	12	21	7	24	34
7OHM,10,11-DHD	7	10	30	16	8	12	—	—	<1	<1	<1	—	—
Phenols	5	3	4	3	5	5	3	30[c]	6	4[d]	4[d]	6	14[c]
No. of components; ratio	3;2:1:2	3;1:2:1	3;1:1:1	3;1:1:1	3;2:2:1	3;4:2:1	3;1:2:2		3;2:2:1	3;?	3;?	3;1:2:2	
7-OHM phenols (No. of components)	3(3)	4(3)	2(3)	ND	1(3)	2(3)	<1(2)	2(2)	2(3)	2(2)	2(2)	1(2)	2(2)

[a] Not detected.
[b] —Not detected or expected.
[c] >95% 4-phenol.
[d] >70% 4-phenol as assessed by uv spectra.
[e] Determination of the ratio of the components not possible because of poor resolution.

TABLE 5

RELATIVE PERCENTAGE OF VARIOUS METABOLITES OBTAINED FROM FLUORO AND METHYL BA ANALOGS IN 3-MC S-10 FRACTION

	DMBA analogs						BA analogs						
Metabolite	DMBA	2F	3F	4F	5F	6F	BA	7M	3F-7M	5F-7M	12M	6F-12M	6F
5,6-DHD	10	5	6	ND[a]	ND	ND	20	17	<1	ND	30	ND	ND
7OHM-5,6-DHD	<1	<1	<1	<1	ND	ND	<1	<1	ND	ND	—[b]	—	—
8,9-DHD	42	32	23	60	72	40	70	25	10	32	37	42	46
7OHM-8,9-DHD	3	21	10	16	3	11	—	8	8	2	—	—	—
10,11-DHD	ND	ND	ND	ND	ND	5	<2	5	25	6	18	32	ND
7OHM-10-11-DHD	9	19	31	16	9	18	—	9	ND	<1	—	—	—
Phenols													
No. of components; ratio	8 3;2:3:2	4 2;3:1	7 2;1:2	2 2;1:1	4 2;2:1	4 2;3:2	2 3;1:1:2	8 3;1:1:1	3 2;1:1	8 3;1:1:1	1 2;1:1	17[c]	29[c]
7-OHM phenols (No. of metabolites)	2(2)	1(3)	1(2)	1(2)	2(4)	2(2)	2(3)	2(2)	2(2)	1(3)	1(2)	2(2)	2(2)

[a] Not detected.
[b] —Not detected or expected.
[c] >90% 4-phenols as assessed by uv spectra.

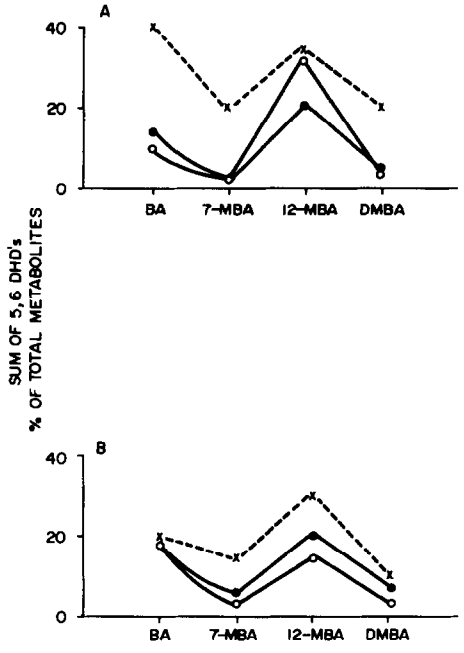


FIG. 1. Production of 5,6-DHDs in induced and uninduced Sprague–Dawley rat liver S-10 fractions. (A) Induction schedule I. (B) Induction schedule II. (X) 3MC induced; (●) β-naphthoflavone induced; (○) phenobarbital induced.

forded 12–14% of this metabolite in β-naphthoflavone-induced and 3MC-induced S-10 fractions (Tables 4 and 5). Similar results were obtained in 3MC-induced S-10 fractions (Table 5). Whereas introduction of a 7-methyl group in BA stimulated the production of the corresponding 10,11-DHD (Table 4), insertion of a 12-methyl function afforded an even greater increase in this D ring metabolism (Figs. 3A and B). In the case of 6F-12MBA, influence of the halogen was again noted; a two-fold increase in 10,11-DHD formation was observed relative to 12MBA.

DISCUSSION

Boyland *et al.* (*27*) concluded that the relative quantities of total metabolites in BA vary with the chemical reactivity of their respective bonds (*28*). This early correlative work has been corroborated using iterative extended Hückel theory (*29a, b*) by Loew *et al.* (*30a, c, d*), and Memory (*30b*).

Since the nucleophilic character of a given bond is a reflection of bond order (*31*), it is not surprising that 1- and 2F-naphthalene undergo Friedel–Crafts acylation at positions 4 and 6 (or 8), respectively (*14*). Furthermore, a number of PAH undergo ozonization (*32*) or reaction with OsO_4 (*33, 34*) preferentially at those positions rich in electrons. Our results provide evidence for both electronic and steric influences on BA analog metabolism. Steric effects seem to be important in

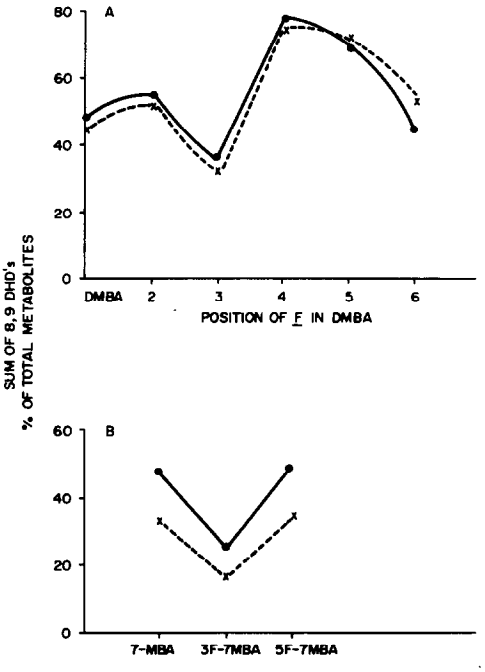


FIG. 2. Production of 8,9-DHDs in induced S-10 fractions (induction schedule II) as a function of F substitution in DMBA (A) and 7MBA (B). (X) 3MC induced; (●) β-naphthoflavone induced.

the production of 5,6-DHDs, since their yield is reduced when positions 4 and 7 are occupied by F or methyl, respectively.

In most discussions of F effects on reactivity attention is paid to the electron-attracting effects of the atom. For example, F-substituted acetic acids are appreciably stronger acids than acetic acid (*35*). However, *p*-fluorophenol has nearly the same acidity as phenol (*36*). This disparity must be a result of donation of electrons from the flourine to the *para* position by a resonance effect. Undoubtedly, introduction of F alters the electron density at various positions in polycyclic aromatic hydrocarbons, but no quantitative studies of such effects have been made (*37*). Furthermore, charge density on the α carbon of naphthalene is stabilized relative to that on the β positions (*38*).

Since the transfer of oxygen from cytochrome *P*-450 (*39, 40*) to a C=C is an electrophilic process (oxenoid mechanism) (*41, 42*), less aromatic rings (*43, 44*) or electron-rich bonds (*28*) in PAH should be more susceptible to oxygen transfer reactions. Intermediate epoxides are predicted to form at positions other than those involving ring junctions, since reaction at the latter positions would result in a greater loss of resonance stabilization in the product. There is considerable evidence to indicate that epoxides are discrete intermediates leading to formation of DHDs upon epoxide hydratase-catalyzed hydration (*45, 46*). Therefore, the resonance contribution of the F electron lone pair to the BA PAH should increase the electron density at that position in the resonance structure which has the greatest possible number of alternating single and double bonds between $\overset{\oplus}{F}$=C

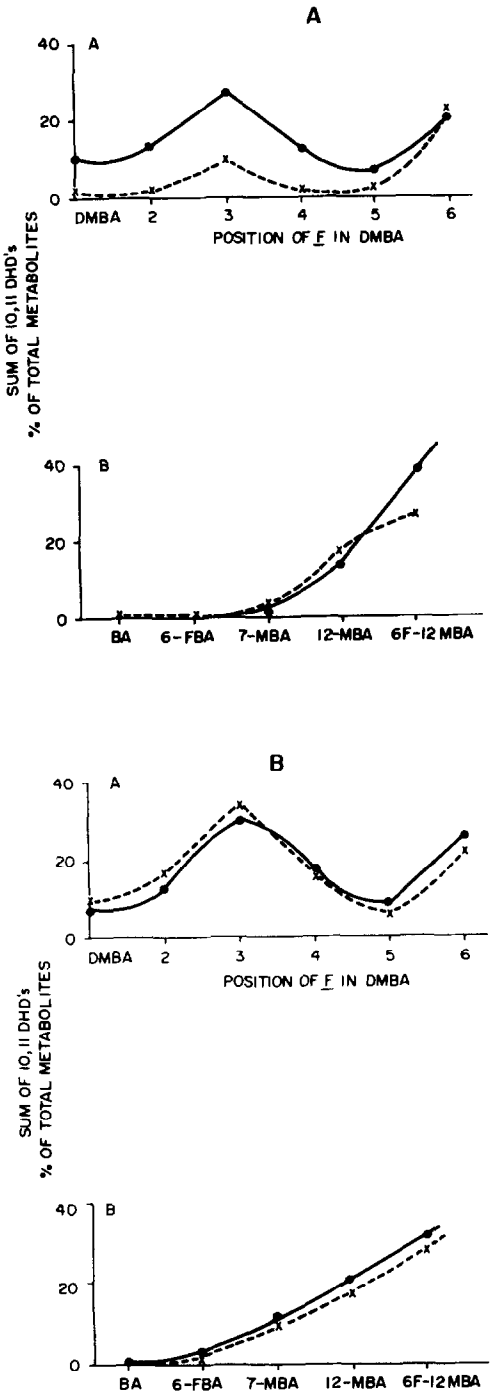
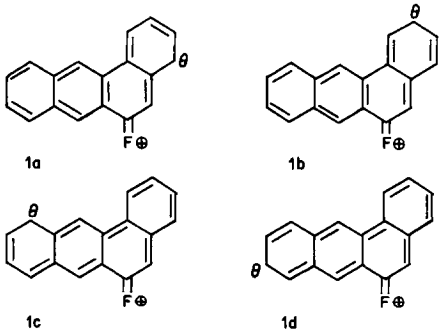
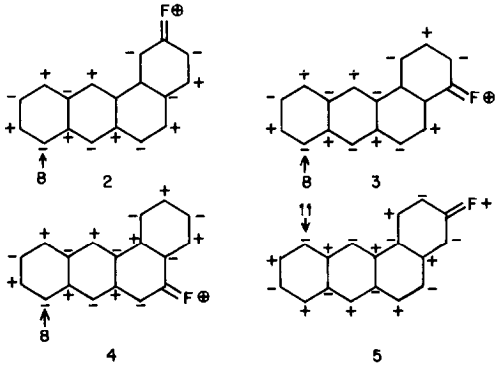


FIG. 3. Production of 10,11-DHDs in induced S-10 fractions as a function of F substitution in DMBA (A) and 7MBA (B). (A) Induction schedule I. (B) Induction schedule II. (X) 3MC induced; (●) β-naphthoflavone induced.

and —$\overset{\theta}{C}$—, wherein —$\overset{\theta}{C}$— is a carbon α in a naphthalene system. Thus, for 6F-BAs (**1**), resonance structure **1a** rather than **1b** correctly predicts enhanced epoxidation and subsequent conversion to phenols and dihydrodiols at the 3,4 position relative to the parent PAH. Resonance structure **1c** rather than **1d** correctly predicts increased formation of the 10,11-DHD metabolite relative to the parent PAH.



Similarly, structures **2**, **3**, and **4** correctly predict high electron density at position 8; and the 8,9-DHD metabolite is preferentially formed from 2F, 4F, and 5F-BA analogs, respectively, relative to parent HCs. Alternatively, 3F-BA analogs (**5**) afford substantially enhanced production of 10,11-DHD relative to the parent HC.



Excluding steric factors this hypothesis serves to explain qualitatively the modulating influence of F substitution on the biofunctionalization of specific double bonds relative of those in the parent PAH. Based on results utilizing the parent PAH and 2- or 3F analogs, electronic effects seem to have little or no effect on K region (5,6 position) dihydrodiol formation. Interestingly, 6F-7MBA (*47*) and 6F-DMBA are potent carcinogens, and in these studies 6F-DMBA yielded enhanced bay-region, 3,4-dihydrodiol production relative to the parent PAH, in the phenobarbital-induced S-10 fraction. In addition to differential biofunctionaliza-

tion of DMBA (*2*) and its F analogs (*1*) in the presence of *P*-450 inhibitors, metabolism studies using reconstituted systems employing purified cytochrome *P*-448 and *P*-450 (*23, 48*) emphasize the complexity of such processes and thus preclude further analysis of the precise events in aryl-HC metabolism. Corroboration of these interpretations, which are based on classical resonance theory, with more sophisticated molecular orbital calculations also is desirable. Nonetheless, we are hopeful that these and similar studies will serve as a basis for predictable alteration of metabolic processes leading to safer biologically significant molecules.

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REFERENCES

1. Y. M. Sheikh, M. N. Inbasekaran, F. B. Daniel, F. D. Cazer, R. W. Hart, and D. T. Witiak, "*Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*" (A. Bjørseth and A. J. Dennis, Eds.), 4th International Symposium, pp. 689–731. Battelle Press, Columbus, Ohio, 1980.
2. Y. M. Sheikh, F. D. Cazer, R. W. Hart, and D. T. Witiak, *Biochem. Biophys. Res. Commun.* **93,** 782–791 (1980).
3. L. H. Botelho, D. E. Ryan, and W. Levin, *J. Biol. Chem.* **254,** 5635–5640 (1979).
4. D. E. Ryan, P. E. Thomas, D. Korzeniowski, and W. Levin, *J. Biol. Chem.* **254,** 1365–1374 (1979).
5. S. A. Atlas and D. W. Nebert, *Arch. Biochem. Biophys.* **175,** 495–506 (1976).
6. F. Oesch, *Xenobiotica* **3,** 305–340 (1973).
7. L. R. Pohl, S. D. Nelson, W. R. Porter, and W. F. Trager, *Biochem. Pharmacol.* **25,** 2153–2162 (1976).
8. S. S. Thorgeirsson, S. A. Atlas, A. R. Boobis, and J. S. Felton, *Biochem. Pharmacol.* **28,** 217–226 (1979).
9. F. B. Daniel, F. D. Cazer, S. M. D'Ambrosio, R. W. Hart, W. H. Kim, and D. T. Witiak, *Cancer Lett.* **6,** 263–272 (1979)
10. F. B. Daniel, L. K. Wong, C. T. Oravec, F. D. Cazer, C'L. A. Wang, S. M. D'Ambrosio, R. W. Hart, and D. T. Witiak, "Polynuclear Aromatic Hydrocarbons; Chemistry Metabolism and Carcinogenesis" (P. W. Jones and P. Leber, Eds.), Vol. 3, pp. 855–883. Ann Arbor Science Pub., Ann Arbor, Mich., 1979.
11. M. S. Newman, D. MacDowell, and S. Swaminathan, *J. Org. Chem.* **24,** 509–512 (1959).
12. M. S. Newman, and A. Tuncay, *J. Org. Chem.* **45,** 348–349 (1980).
13. T. J. Slaga, G. L. Gleason, J. DiGiovani, D. L. Berry, M. R. Juchau, P. P. Fu, K. B. Sukumaron, and R. G. Harvey, "Polynuclear Aromatic Hydrocarbons; Chemistry, Metabolism, and Carcinogenesis" (P. W. Jones and P. Leber, Eds.), Vol. 3, pp. 753–764. Ann Arbor Science Pub., Ann Arbor, Mich., 1979.
14. Y. M. Sheikh, F. D. Cazer, R. W. Hart, and D. T. Witiak, *J. Org. Chem.* **44,** 3715–3717 (1979).
15. M. S. Newman, L. E. Fikes, M. M. Hashem, R. Kannan, and V. Sankaran, *J. Med. Chem.* **21,** 1076–1078 (1978).
16. W. Girke and E. D. Bergman. *Chem. Ber.* **109,** 1038–1045 (1978).

17. J. A. BOND, H.-Y. L. YANG, M. W. MAJESKY, E. P. BENDITT, AND M. R. JUCHAU, *Tox. Appl. Pharmacol.* **52,** 323–335 (1980).
18. A. D. MACNICOLL, P. M. BURDEN, O. RIBEIRO, A. HEWER, P. L. GROVER, AND P. SIMS, *Chem. Biol. Interact.* **26,** 121–132 (1979).
19. M. NORDQUIST, D. R. THAKKER, W. LEVIN, H. YAGI, D. E. RYAN, P. E. THOMAS, A. H. CONNEY, AND D. M. JERINA, *Mol. Pharmacol.* **16,** 643–655 (1979).
20. E. BOYLAND AND P. SIMS, *Biochem. J.* **97,** 7–16 (1965).
21. E. BOYLAND AND P. SIMS, *Biochem. J.* **91,** 493–506 (1964).
22. E. BOYLAND AND F. WEIGERT, *Brit. Med. Bull.* **4,** 354–359 (1947).
23. D. R. THAKKER, W. LEVIN, H. YAGI, D. RYAN, P. E. THOMAS, J. M. KARLE, D. E. LEHR, D. M. JERINA, AND A. H. CONNEY, *Mol. Pharmacol.* **15,** 138–153 (1979).
24. B. TIERNEY, A. HEWER, A. D. MACNICOLL, P. G. GERVASI, H. RATTLE, C. WALSH, P. L. GROVER, AND P. SIMS, *Chem. Biol. Interact.* **23,** 243–257 (1978).
25. M. S. NEWMAN, "Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis" (R. I. Freudenthal and P. W. Jones, Eds.), Vol. 1, pp. 203–207. Raven Press, New York, 1976.
26. M. S. NEWMAN, R. G. MENTZER, AND G. SLOMP, *J. Amer. Chem. Soc.* **85,** 4018–4020 (1963).
27. E. BOYLAND, *Brit. Med. Bull.* **20,** 121–126 (1964).
28. J. C. ARCOS AND M. F. ARGUS, "Advances in Cancer Research" (A. Haddow and S. Weinhous, Eds.), Vol. 11, pp. 305–471. Academic Press, New York, 1968.
29a. M. ZERNER AND M. GOUTERMAN, *Theor. Chim. Acta* **4,** 44–63 (1966).
b. M. ZERNER, M. GOUTERMAN, AND H. KOBAYASHI, *Theor. Chim. Acta* **6,** 363–400 (1966).
30a. G. H. LOEW, J. PHILLIPS, J. WONG, L. HJELMELAND, AND G. PACK, *Cancer Biochem. Biophys.* **2,** 113–133 (1978).
b. J. D. MEMORY, *Int. J. Quantum Chem.* **15,** 363–368 (1979).
c. G. H. LOEW, B. S. SUDHINDRA, AND J. E. FERRELL JR., *Chem.-Biol. Interact.* **26,** 75–89 (1979).
d. G. LOEW, M. POULSON, J. FERRELL, AND D. CHAET, *Chem.-Biol. Interact.* **31,** 319–340 (1980).
31. C. A. COULSON, "Advances in Cancer Research" (J. P. Greenstein and A. Haddow, Eds.), Vol. 1, pp. 1–56. Academic Press, New York, 1953.
32. F. T. WALLENBERGER, *Tetrahedron Lett.* (9), 5–9 (1959).
33. G. M. BADGER, *J. Chem. Soc.*, 456–463 (1949).
34. G. M. BADGER, *J. Chem. Soc.*, 1809–1814 (1950).
35. Z. RAPPOPORT (Ed.), "CRC Handbook of Tables for Organic Compound Identification," 3rd ed., Table XXVII, pp. 429–433. Chemical Rubber Co., Cleveland, Ohio, 1967.
36. Z. RAPPOPORT, ibid., Table XXVIII, p. 434.
37. G. A. OLAH AND Y. K. MO, "Advances in Flourine Chemistry" (J. C. Tatlow, R. D. Peacock, H. H. Hyman, and M. Stacy, Eds.), Vol. 7, pp. 69–112. Butterworths, London, 1973.
38. A. STREITWEISER, "Molecular Orbital Theory for Organic Chemists," Chap. 14, pp. 413–431. Wiley, New York, 1961.
39. R. KATO, *Pharmacol. Ther.* **6,** 41–98 (1979).
40. J. P. WHITLOCK, JR., AND H. V. GELBOIN, *Pharmacol. Ther.* **4,** 587–599 (1979).
41. G. A. HAMILTON, "Molecular Mechanism of Oxygen Activation" (O. Hayashi, Ed.), pp. 405–451. Academic Press, New York, 1974.
42. R. E. WHITE AND M. J. COON, *Annu. Rev. Biochem.* **49,** 315–356 (1980).
43. M. RANDIC, *Tetrahedron* **31,** 1477–1481 (1975).
44. S. C. SHARMA, *Z. Phys. Chem. Leipzig* **259,** 1031–1036 (1978).
45. F. OESCH, P. BENTLEY, AND H. R. GLATT, *Int. J. Cancer* **18,** 448–452 (1976).
46. A. W. WOOD, W. LEVIN, A. Y. H. LU, H. YAGI, O. HERNANDEZ, D. M. JERINA, AND A. H. CONNEY, *J. Biol. Chem.* **251,** 4882–4890 (1976).
47. J. L. STEVENSON AND E. VON HAAM, *Amer. Ind. Hyg. Assoc. J.* **26,** 475–478 (1965).
48. S. K. YANG, M. W. CHOU, P. G. WISLOCKI, AND A. Y. H. LU, "Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects" (A. Bjørseth and A. J. Dennis, Eds.), pp. 733–752. Fourth International Symposium, Battelle Press, Columbus, Ohio, 1980.