Synthesis and Mutagenicity of Modified Chrysenes Related to the Carcinogen, 5-Methylchrysene[1]

Stephen S. Hecht,* Myrna Loy, Robert Mazzarese, and Dietrich Hoffmann

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595.
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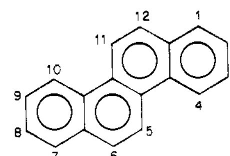
The environmental carcinogen, 5-methylchrysene (1), is more carcinogenic on mouse skin and more mutagenic toward *Salmonella typhimurium* than any of the other methylchrysene isomers or chrysene and has activity comparable to benzo[*a*]pyrene. To investigate the modes of metabolic activation of 1, a series of modified chrysene derivatives was synthesized. The following compounds were prepared: 1-fluoro-5-methylchrysene (1-F-5-MeC, **2**), 3-F-5-MeC (**3**), 6-F-5-MeC (**4**), 7-F-5-MeC (**5**), 9-F-5-MeC (**6**), 11-F-5-MeC (**7**), 12-F-5-MeC (**8**), 1-fluoro-4-methylchrysene (**9**), 6-methoxy-5-methylchrysene (**10**), 12-methoxy-5-methylchrysene (**11**), and 5-methoxychrysene (**12**). Compounds **2**, **3**, and **5–12** were prepared by photocyclization of the appropriate alkene precursors, which, in turn, were prepared by either Grignard reactions and dehydration (**5–8**, **11**), Wittig reactions (**2**, **3**, **9**), or enol ether formation (**10**, **12**). 6-F-5-MeC (**4**) was prepared by reaction of 5,6-dihydro-5,6-dihydroxy-5-methylchrysene with diethylaminosulfur trifluoride. All compounds were assayed for mutagenicity toward *S. typhimurium* strain TA-100. Fluoro derivatives **2**, **3**, and **8** were less mutagenic than 1, whereas **4**, **6**, and **7** were as mutagenic; **5** was toxic to the bacteria. Methoxy compounds **10–12** were less mutagenic than 1, as was 5,12-dimethylchrysene; **9** was more mutagenic than 4-methylchrysene. These results indicated that the 12, 1, and 3 positions of 1 were involved in metabolic activation and the 6, 9, and 11 positions were not; the nonplanarity of 1 apparently contributes to its mutagenic activity. A 1,2-dihydrodiol 3,4-epoxide may be the ultimate mutagen derived from 1 and the adjacent 12-peri position may be involved in its formation.

The environmental carcinogen, 5-methylchrysene (**1**), is more tumorigenic on mouse skin and more mutagenic toward *Salmonella typhimurium* than any of the other five methylchrysene isomers or chrysene.[2-6] This phenomenon of increased carcinogenicity upon substitution of a methyl group at the appropriate position is often observed for polynuclear aromatic hydrocarbons (PAH). Thus, while chrysene is at most only weakly carcinogenic, **1** has carcinogenic activity comparable to benzo[*a*]pyrene (BaP). These observations encouraged us to begin a detailed study of the structural features required for carcinogenicity in the methylchrysene series. It is hoped that the conclusions reached from these studies will allow prediction of carcinogenic activity for methylated PAH.

The carcinogenic activity of PAH is a result of conversion in vivo to electrophilic species (ultimate carcinogens) which can react with nucleophilic cellular macromolecules. The formation of the ultimate carcinogen (metabolic activation) competes with metabolic detoxification processes. For BaP, major pathways of metabolism include oxidation at the 3 and 6 positions; however, the ultimate carcinogen is apparently a 7,8-dihydrodiol 9,10-epoxide.[7-10] In order to probe the involvement of the various positions of **1** in metabolic activation, a series of fluorinated methylchrysene derivatives was synthesized. The major effect of substituting an electron-withdrawing fluorine for hydrogen is expected to be decreased carcinogenicity or mutagenicity of an *x*-fluoro-5-methylchrysene (e.g., **2–8**) if position *x* is involved in metabolic activation. Fluorine substitution could also influence electron density at other ring positions. Fluorine has been used as a probe in studies of other chemical carcinogens, including 2-acetylaminofluorene and 7-methylbenz[*a*]anthracene.[11,12] Electron-releasing methoxy groups (compounds **10–12**) would also be expected to influence carcinogenicity or mutagenicity, but the effects may not be as clear because of possible detoxification reactions by OCH_3 cleavage and steric factors. In this report, we describe the synthesis and mutagenic activity of the modified methylchrysenes **2–12**; preliminary results of bioassays for tumor-initiating activity are also described.



1, 5-CH_3
2, 1-F-5-CH_3
3, 3-F-5-CH_3
4, 6-F-5-CH_3
5, 7-F-5-CH_3
6, 9-F-5-CH_3
7, 11-F-5-CH_3
8, 12-F-5-CH_3
9, 1-F-4-CH_3
10, 6-MeO-5-CH_3
11, 12-MeO-5-CH_3
12, 5-MeO

Synthesis. The syntheses of fluoro-5-methylchrysenes **5–8** and 12-methoxy-5-methylchrysene (**11**) are summarized in Scheme I. The use of this method for synthesis of **1** will be described in detail elsewhere. The acetonaphthones were commercially available except for 3-fluoroacetonaphthone which was prepared by reaction of 3-fluoronaphthoic acid with methyllithium.

The alkenes **18–22** were obtained as mixtures of cis and trans isomers. In each case, the isomeric olefins **13–17** were also formed and consisted of 28–61% of the product mixtures. The formation of **13–17** was demonstrated both by NMR (terminal methylene protons between 5.00 and 5.25 ppm) and by combined GLC–MS (the cis–trans isomers of **18–22** and the olefins **13–17** were cleanly separated and the latter showed base peaks corresponding to loss of the benzyl radical in contrast to **18–22**).

The relatively large amounts of **13–17** may have resulted from an unfavorable steric interaction in **18–22**, in which the methyl group can prevent coplanarity of the naphthyl and phenyl moieties. Since **13–17** may not have undergone photocyclization to the desired chrysenes, the use of the Wittig reaction was also studied. While the Wittig reaction was suitable for use in the synthesis of **1**,[13] difficulties were encountered in the preparation of alkenes **20** and **22**; the Grignard sequence was generally better.

The alkenes **18–22** were photolytically cyclized[14] to the corresponding chrysenes **5–8** and **11**. This reaction was also used in the preparation of **2**, **3**, **9**, **10**, and **12**. The reactions were followed by GLC until formation of the chrysenes was complete. The rates of ring closure varied

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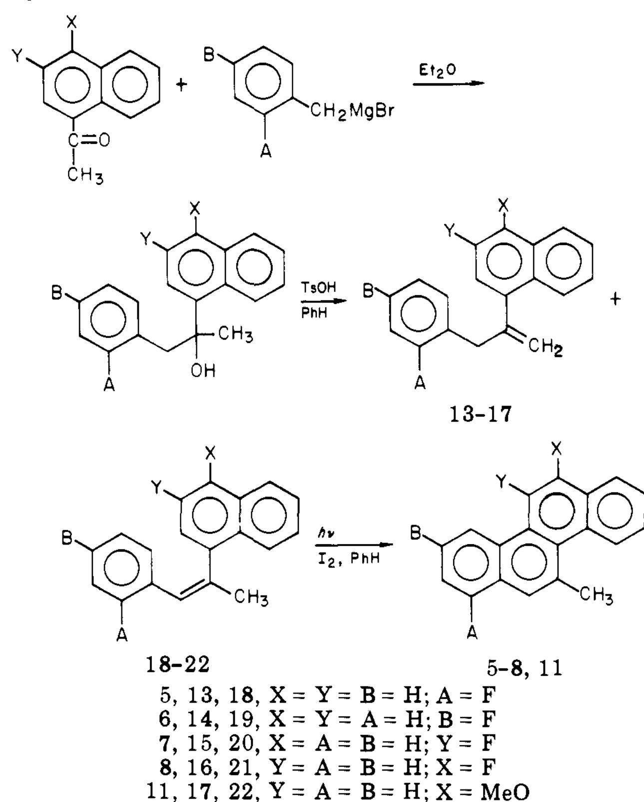
Table I. Synthesis and Properties of Substituted Chrysenes

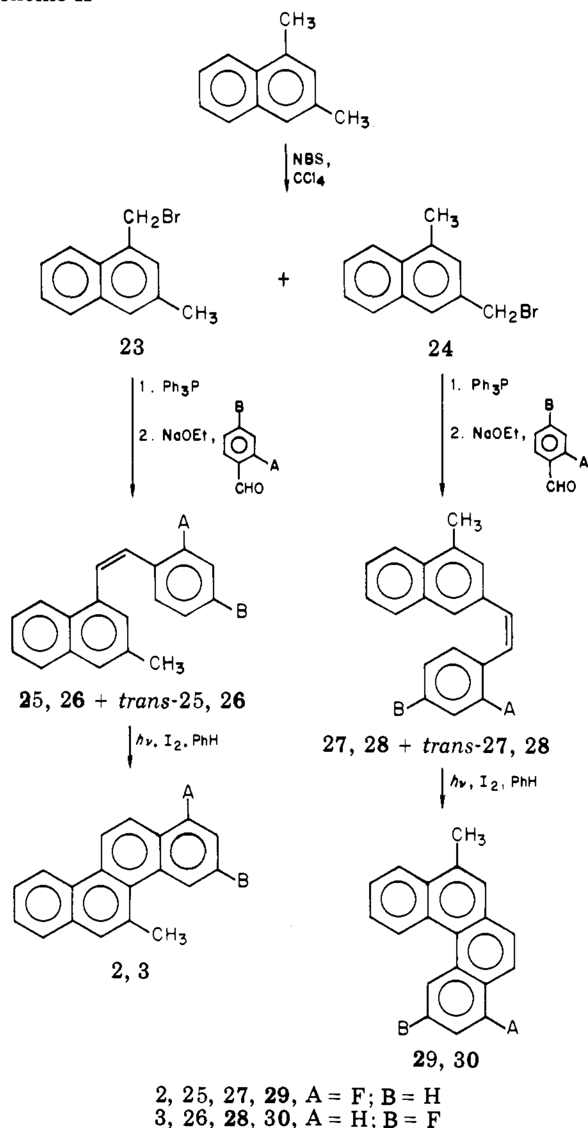
Compd	Synthetic scheme	Recrystn solvent	Mp, °C	Analyses[a]	UV (hexane), λ_{max} (ϵ)	Formation by photolysis	
						Time, h[b]	% yield[c]
2	2	EtOH	97-97.5	C, H, F	269 (96700)	1.0	6.4
3	2	EtOH	119.5-120.5	C, H, F	269.5 (109400)	0.5	19.0
4	6	EtOH	103.5-104	C, H, F	269 (108700)		
5	1	EtOH	114.5-115	C, H, F	271 (81200)	12.0	8.9
6	1	EtOH	113-114	C, H, F	268 (84300)	4.5	13.1
7	1 + 6	EtOH	97-98	C, H, F	266 (94200)	4.5	7.2
8	1	95% EtOH	77.5-79	C, H, F	268.7 (129600)	6.0	29.7
9	3	95% EtOH	118-119	C, H, F	270 (141800)	1.5	77.0
10	4	95% EtOH	98-99	C, H	271.5 (112700)	4.0	3.2
11	1	EtOH	119-120	C, H	272 (87700)	3.8	36.7
12	4	95% EtOH	144-145[d]		265.5 (70000)	16.0	15.0

[a] Analyses listed were within 0.3% of calculated values. Analyses were obtained on new compounds only. [b] Total time of photolysis. Reactions were followed by GLC until formation of chrysenes was maximized relative to other products. See Experimental Section for details. [c] Isolated yields of highly purified material (>99.9%) suitable for bioassay. [d] Lit.[18] 142-143 °C.

Scheme I



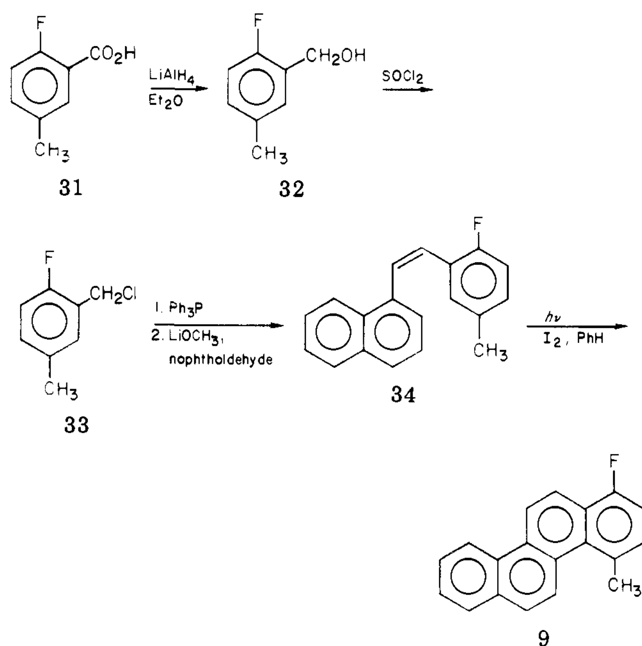
Scheme II



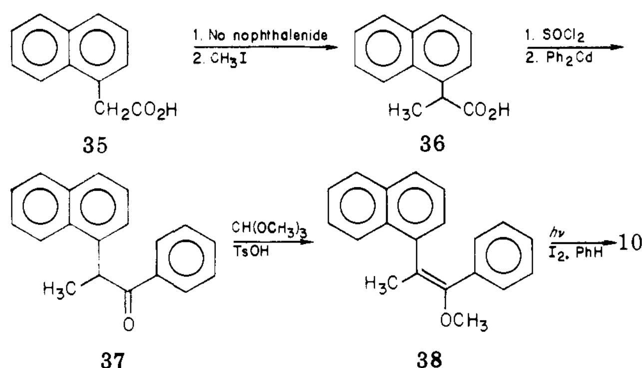
widely (0.5–16 h for completion) as indicated in Table I. Products, which were formed in 13–84% yield, were purified by silica gel chromatography followed by recrystallization. The yields of highly purified material, suitable for bioassay, are given in Table I. The criteria for purity were demanding, since contamination of a nonactive compound by even trace amounts of a highly carcinogenic compound, such as **1**, could lead to incorrect results. In each case, chromatographic properties (GLC, HPLC, and TLC), elemental analyses, melting points, and spectral characteristics (IR, NMR, MS, and UV) indicated purity greater than 99.9%. These criteria were used for all compounds **1**–**12**.

The syntheses of 1-fluoro-5-methylchrysene (**2**) and 3-fluoro-5-methylchrysene (**3**) are summarized in Scheme II. 1-Bromomethyl-3-methylnaphthalene (**23**) was synthesized by reaction of NBS with 1,3-dimethylnaphthalene. This gave a mixture of **23** and 3-bromomethyl-1-methylnaphthalene (**24**) which was partially separated by silica gel chromatography. Compounds **23** and **24** were distinguished from each other by NMR; the *peri*-methyl group of disubstituted naphthalenes absorbs further downfield than methyl groups in the 2 and 3 positions.[15] Thus in **23** the methyl resonance was observed at 2.55 ppm and in **24** at 2.72 ppm. The phosphonium salts obtained from the fractions enriched in **23** were allowed to react with

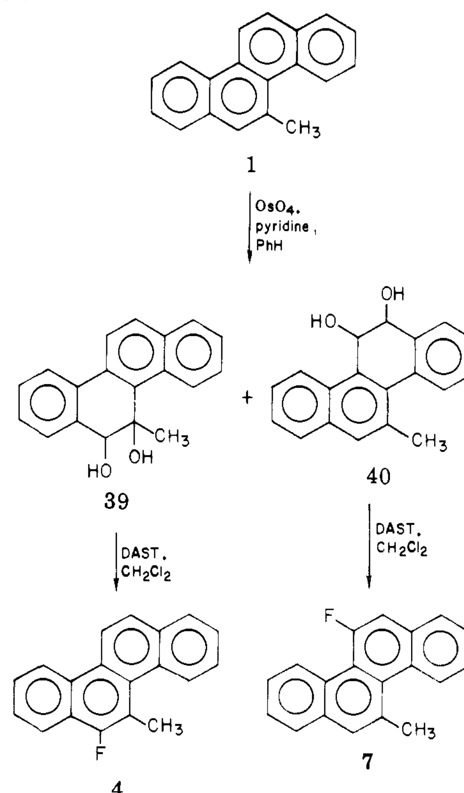
Scheme III



Scheme IV



Scheme V



either *o*-fluorobenzaldehyde or *p*-fluorobenzaldehyde to give mixtures of cis and trans isomers of alkenes **25** or **26**, respectively. At this point, the *cis*- and *trans*-alkene mixtures (**25** or **26**) were further purified by column chromatography to remove most of **27** or **28** resulting from Wittig reaction of the phosphonium salt derived from **24**. Photolyses of **25** or **26** gave 1-fluoro-5-methylchrysene (**2**) or 3-fluoro-5-methylchrysene (**3**), respectively, which were purified by silica gel chromatography and recrystallization. The contaminating olefins **27** (in the case of **25**) and **28** (in the case of **26**) could have given rise to either fluorinated methylbenz[*a*]anthracenes or methylbenzo[*c*]phenanthrenes. Since the former may have been difficult to remove from **2** or **3**, each cis and trans isomer of **27** or **28** was isolated by preparative GLC and photocyclized. These alkenes gave exclusively products which were well separated from **2** or **3** by GLC and were easily removed from **2** or **3** by column chromatography. These products **29** and **30** are believed to be benzo[*c*]phenanthrenes rather than benz[*a*]anthracenes by comparison of their UV spectra to the parent compounds and by their GLC retention times; the methylbenz[*a*]anthracene isomers would have been expected to coelute with **2** or **3**.

The syntheses of 1-fluoro-4-methylchrysene (**9**) and 6-methoxy-5-methylchrysene (**10**) are summarized in Schemes III and IV, respectively. A method similar to the latter was used for the preparation of 5-methoxychrysene (**12**), starting with benzyl 1-naphthyl ketone. The synthesis of 6-fluoro-5-methylchrysene (**4**) was not done by the photochemical route because preparation of the precursor alkene was difficult. In an attempt to prepare this alkene, benzyl 1-naphthyl ketone was brominated to give α-bromobenzyl 1-naphthyl ketone and the bromine was replaced by F to give the corresponding α-fluoro ketone. However, attempted conversions of this substance to the corresponding tertiary alcohol using various methyl organometallics failed, due to facile enolization. Therefore, the synthesis of **4** was accomplished by an alternate route, as summarized in Scheme V.

5-Methylchrysene (**1**) was allowed to react with OsO_4 and pyridine in benzene, under conditions generally used for conversion of PAH to the corresponding K-region *cis*-diols.[16] The reaction proceeded sluggishly to give a mixture of **39** and **40** (ratio of **39**:**40** = 4:1; overall yield of **39** and **40**, 69%). The diols were separated by column chromatography on silica gel and were identified by spectral and analytical properties. The 5,6-diol **39** was distinguished from the 11,12-diol **40** by its NMR spectrum (H-11 and H-12 of **40**, doublets 4.82 and 5.28 ppm; H-6 of **39**, singlet 5.15 ppm; 5-CH_3 of **40**, singlet 2.75 ppm; 5-CH_3 of **39**, singlet 1.40 ppm).

Reaction of **39** with 1 equiv of diethylaminosulfur trifluoride (DAST[17]) at −78 °C in CH_2Cl_2 gave a mixture of products from which **4** was isolated in 20% yield by column chromatography. Formation of **4** resulted from replacement of the 6-OH by −F and dehydration.

When the 11,12-diol **40** was allowed to react with 1 equiv of DAST, a mixture was obtained, from which the fluorinated methylchrysene product was easily separated by column chromatography. Interestingly, only 11-fluoro-5-methylchrysene (**7**) was formed; the other expected product, 12-fluoro-5-methylchrysene (**8**), was not detected in the reaction mixture. This was determined by GLC, under conditions which separated **7** and **8**. This may reflect preferential formation of the carbonium ion at the 11 position and warrants investigation in other systems. The reaction of DAST with **40** is not the best method to prepare **7** because **40** is relatively difficult to obtain.

Table II. Mutagenic Activity of Chrysene Derivatives to *S. typhimurium* (TA-100)

Compd	HIS⁺, revertants/plate[a]
7-Fluoro-5-methylchrysene (**5**)	254 ± 42
12-Fluoro-5-methylchrysene (**8**)	354 ± 8
3-Fluoro-5-methylchrysene (**3**)	393 ± 36
1-Fluoro-5-methylchrysene (**2**)	423 ± 46
5-Methylchrysene (**1**)[b]	521 ± 67
11-Fluoro-5-methylchrysene (**7**)	531 ± 92
9-Fluoro-5-methylchrysene (**6**)	633 ± 120
6-Fluoro-5-methylchrysene (**4**)	679 ± 141
4-Methylchrysene[c]	266 ± 30
1-Fluoro-4-methylchrysene (**9**)	486 ± 74
6-Methoxy-5-methylchrysene (**10**)	146 ± 54
12-Methoxy-5-methylchrysene (**11**)	139 ± 21
5-Methoxychrysene (**12**)	189 ± 9
5,12-Dimethylchrysene[d]	159 ± 8
Dimethyl sulfoxide	157 ± 36

[a] In each case, 20 μg of test compound in 10 μL of Me_2SO was added to the plate with 200 μL of microsomal suspension (33 μg/μL of protein). Each value is the average of six replicate plates. [b] M. S. Newman, *J. Am. Chem. Soc.*, 62, 870 (1940). [c] W. E. Bachmann and W. S. Struve, *J. Org. Chem.*, 4, 456 (1939). [d] D. Cagniant and M. M. Delēphine, *C. R. Hebd. Seances Acad. Sci.*, 256, 5590 (1963).

Mutagenic Activity. Mutagenicity was assayed in *Salmonella typhimurium* strain TA-100[19] with addition of the S-9 fraction obtained from livers of Arochlor induced rats. The results are summarized in Table II. Each modified methylchrysene derivative was tested at a dose (20 μg/plate) equivalent to the maximum response for 5-methylchrysene. No significant toxicity was observed, except with 7-fluoro-5-methylchrysene (**5**).

Among the fluorinated 5-methylchrysenes, 7-fluoro-5-methylchrysene (**5**) caused the least number of reversions/plate; however, because of the toxicity of this compound its actual mutagenic activity was not easily judged. 12-Fluoro-5-methylchrysene (**8**), 3-fluoro-5-methylchrysene (**3**), and 1-fluoro-5-methylchrysene (**2**) were all qualitatively less mutagenic than 5-methylchrysene whereas 11-fluoro-5-methylchrysene (**7**), 9-fluoro-5-methylchrysene (**6**), and 6-fluoro-5-methylchrysene (**4**) were as mutagenic or more mutagenic than 5-methylchrysene toward this strain of *S. typhimurium*. These results indicate that the 12 position, the 1 position, and the 3 position of **1** are involved in metabolic activation to the ultimate bacterial mutagen, whereas the 6, 9, and 11 positions do not participate in metabolic activation. The role of the 7 position cannot be clearly assessed because of the rather high toxicity of **5**.

The tumor-initiating activity on mouse skin of the fluorinated methylchrysene derivatives is being tested in a parallel study, the results of which will be reported separately. Current data indicate a similar trend in those experiments. The least active compound was 3-fluoro-5-methylchrysene (**3**); 1-fluoro-5-methylchrysene (**2**) and 12-fluoro-5-methylchrysene (**8**) were also less active than **1**, whereas the other fluoro compounds, **4**–**7**, were as tumorigenic as **1**. Thus, with the exception of 7-fluoro-5-methylchrysene (**5**), the general trends of mutagenicity and tumor-initiating activity were similar in these experiments.

The mutagenic activity of 1-fluoro-4-methylchrysene (**9**) was greater than that of 4-methylchrysene. This implies that conversion of 4-methylchrysene to a nonmutagenic metabolite may have been blocked by fluorine substitution and suggests that a nonplanar configuration, which is common to 4-methylchrysene and **9**, may play a role in the mutagenic activity of **1** and **9**. This nonplanarity, which results from an unfavorable interaction between the 4-methyl and 5-hydrogen or vice versa, amounts to 10–15 °C in the case of 5,12-dimethylchrysene, for which x-ray crystallographic data has been obtained.[20] While nonplanarity is clearly not a sufficient condition for mutagenicity, it is likely to be important since a number of carcinogenic and mutagenic PAH, including 7,12-dimethylbenz[*a*]anthracene, have this configuration.[6,21] While the mutagenicity of **9** exceeds that of 4-methylchrysene, the tumor-initiating activity of **9** was not significantly greater than that of 4-methylchrysene.[4,5]

The results of the mutagenicity assays for the methoxy derivatives **10**–**12** and for 5,12-dimethylchrysene indicate that all four of these compounds are significantly less potent mutagens than **1**. Bioassays of these compounds as tumor initiators have also been completed. The only one showing significant tumor-initiating activity on mouse skin was **12** which was slightly less active than **1**. The other compounds were inactive at the doses studied.[4,5,22] The lower mutagenic and tumor-initiating activities of **11** and of 5,12-dimethylchrysene in which the 12 position was sterically blocked are in agreement with the decreased activity of **8**, further indicating that the 12 position of **1** is involved in metabolic activation to the ultimate mutagen. The reasons for the diminished activity of **10** and **12** are not presently known. Demethylation of the methoxy group of **12** to give the corresponding chrysenol was not observed to any significant degree in a preliminary study of the in vitro metabolism of **12**.

These mutagenicity data indicate that the metabolic activation of 5-methylchrysene to the ultimate mutagen involves the 12 position, 1 position, and 3 position; the 6, 9, and 11 positions are apparently not involved. Whereas 5-methylchrysene has two K regions (5,6 and 11,12), two bay regions (4,5 and 10,11), two peri positions (6 and 12), and two non-K regions (1–4 and 7–10), only half the molecule (12, 1–4) appears to be involved in metabolic activation. This implies that a sterically unhindered peri position (the 12 position) may be necessary for formation of a mutagenic metabolite in the adjacent 1–4 positions. It is possible that this ultimate mutagen derived from 5-methylchrysene is a 1,2-dihydrodiol 3,4-epoxide by analogy to results for BaP; a 7,8-dihydrodiol 9,10-epoxide is apparently not an activated form of **1**. The syntheses of these diol epoxides and further metabolic studies are currently in progress.

Experimental Section

All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were run on a Perkin-Elmer Model 267 spectrometer in Nujol mulls (solids) or as liquid films. ^1H NMR spectra were determined with a Hitachi Perkin-Elmer Model R-24 spectrometer in $CDCl_3$ solution with Me_4Si as internal reference. All compounds gave ^1H NMR signals compatible with their assigned structures; only the spectra of the chrysenes **1**–**12** and of intermediates in which ^1H NMR spectra were necessary for structure determination are given. UV spectra were run on a Cary Model 118 spectrometer in spectroquality hexane. Mass spectra and combined GLC–mass spectra were recorded with a Hewlett-Packard Model 5982A mass spectrometer. Gas–liquid chromatography was done with a Hewlett-Packard Model 5711 instrument equipped with a flame ionization detector and column A (8 ft × $^1/_8$ in., 10% OV-17 on gas-chrom Q, 80–100 mesh) or column B (20 ft × $^1/_8$ in., 10% OV-17 on gas-chrom Q, 80–100 mesh) with a flow rate of 40 mL/min of He. TLC was done with 0.25-mm silica gel 60 F_{254} (Merck) glass plates. High-pressure liquid chromatography was performed with a Waters Associates Model ALC/GPC-202 high-speed liquid chromatograph equipped with a Model 6000A solvent delivery system, a Model 660 solvent programmer, a Model U6K septumless injector, a Model 440 UV/visible detector, and

a 6 mm × 30 cm microbondapak/C_{18} column. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn., and were within ±0.3% of the calculated values.

3′-Fluoro-1′-acetonaphthone. To a stirred solution of 4.5 g (0.024 mol) of 3-fluoro-1-naphthoic acid[23] in 50 mL of ether under nitrogen was added a solution of 30.7 mL of 1.7 M methyllithium in 25 mL of ether. The reaction mixture was stirred for 4 h and then quenched with H_2O. The ether layer was washed (saturated $NaHCO_3$ and H_2O), dried ($MgSO_4$), and concentrated to give a residue which was chromatographed on silica gel with elution by 50:50 hexane–$CHCl_3$. The product (4.2 g, 92%) was a pale yellow oil: IR (film) 1680 cm^{-1} (C=O); MS *m/e* (rel intensity) 188 (17), 173 (41), 146 (15), 145 (100). This material was used directly in the preparation of **20**.

1-(4-Fluorophenyl)-2-(1-naphthyl)propene (19). This procedure is illustrative of those used for preparation of **18–22**. *p*-Fluorobenzylmagnesium bromide was prepared by the addition of 5 g (0.026 mol) of *p*-fluorobenzyl bromide (Fairfield Chemical Co.) in 75 mL of anhydrous Et_2O to 0.96 g (0.04 mol) of Mg in 100 mL of Et_2O under N_2 in the usual manner. The mixture was stirred at room temperature for 1 h after formation seemed complete and then refluxed for 2 h. After cooling the Grignard reagent, a solution of 5.31 g (0.03 mol) of 1-acetonaphthone (Aldrich Chemical Co.) in 60 mL of Et_2O was added dropwise. Stirring was continued at room temperature for 0.5 h; the mixture was refluxed for 5 h and then allowed to stand overnight. Additional aqueous NH_4Cl (50 mL) was added cautiously and the layers were separated. The aqueous layer was extracted with Et_2O, and the combined Et_2O solutions were washed three times with H_2O, dried ($MgSO_4$), and evaporated. The oily residue (8 g) was chromatographed on 400 g of silica gel eluting with 4:1 hexane–$CHCl_3$ to give 4.4 g (60%) of crude 1-(4-fluorophenyl)-2-(1-naphthyl)propan-2-ol: clear liquid; IR 3422 cm^{-1} (br, OH). This was used without further purification in the next step. A solution of 2.57 g (9.2 mmol) of the above crude alcohol and a catalytic amount of *p*-toluenesulfonic acid in 200 mL of dry benzene was refluxed overnight using a Dean-Stark trap. The solvent was evaporated in vacuo leaving 2.4 g of crude product. This was chromatographed on 120 g of silica gel with elution by hexane to give 1.6 g (70%) of product as a mixture of **19** (cis and trans) and 14, according to analysis by GLC–MS (column A, 250 °C); the *exo*-methylene isomer 14 (28%) eluted in 4.0 min and gave MS *m/e* (rel intensity) 262 (16), 153 (100), 152 (47); the cis and trans isomers of **19** eluted at 3.5 and 5.5 min and gave identical mass spectra [*m/e* (rel intensity) 262 (87), 247 (100)]. Similar data and yields were obtained for **18** and **20–22**. Anal. ($C_{19}H_{15}F$) C, H, F. Anal. ($C_{20}H_{18}O$) C, H.

Reaction of 1,3-Dimethylnaphthalene with NBS. A mixture of 5.0 g (0.032 mol) of 1,3-dimethylnaphthalene (Aldrich Chemical Co., pure by GLC analysis[24]) and 5.7 g (0.032 mol) of *N*-bromosuccinimide in 125 mL of CCl_4 was refluxed for 1 h with stirring until all of the NBS was reacted and succinimide was floating. After cooling, the mixture was filtered and the filtrate evaporated. The residue was chromatographed through a silica gel column, eluting with hexane. This gave 3.0 g (40%) of monobromo products [MS *m/e* (rel intensity) 236 (11), 234 (11), 155 (100), 156 (14), 153 (20), 152 (14)] in a ratio of 2:1 of the 1-bromomethyl compound **23** to the 3-bromomethyl isomer **24** by NMR. The bromo product was rechromatographed on silica gel with elution by hexane to partially separate the isomers. The 3-bromomethyl analogue **24** eluted first, followed by the desired 1-bromomethyl-3-methylnaphthalene (**23**): NMR of **23** ($CDCl_3$) 2.45 (3 H, s, CH_3), 4.85 ppm (2 H, s, CH_2Br); NMR of **24** ($CDCl_3$) 2.60 (3 H, s, CH_3), 4.55 ppm (2 H, s, CH_2Br).

1-(4-Fluorophenyl)-2-(3-methyl-1-naphthyl)ethylene (26) and 25. A solution of **23** (contaminated with 40% of **24**) (3.0 g, 0.013 mol) and 9.2 g (0.035 mol) of triphenylphosphine in 125 mL of xylene was refluxed for 2 h and cooled. Filtration gave 5.4 g of the quaternary salts.

To a stirred solution of 0.5 g (0.001 mol) of phosphonium salt and 0.124 g (0.001 mol) of *p*-fluorobenzaldehyde (Aldrich Chemical Co.) in 15 mL of EtOH was added a solution of 0.001 mol of NaOEt (0.023 g of Na in 10 mL of absolute EtOH). This was stirred at room temperature for 2 h and then the solvent was evaporated. The residue was triturated with hexane to remove triphenylphosphine oxide and then chromatographed on silica gel with elution by hexane. Elution of the cis and trans isomers of **26** was followed by GLC (column A, 250 °C). Since the starting **23** was contaminated with **24**, four products (the cis–trans isomers of **26** and **28**) were observed, with relative retention times of 1.0, 1.4, 2.5, and 3.4. All four compounds showed *m/e* 262 (M^+). To identify the desired isomers (**26**), each peak was collected, dissolved in benzene with a trace of iodine in a 1-cm UV cell, and irradiated with the 250-W medium-pressure lamp. Only the peaks of relative retention time 1.0 and 2.5 gave rise to the desired chrysene **3**, as determined by GLC (column A, 250 °C, retention time 25.5 min) and UV. The peaks of relative retention time 1.4 and 3.4 gave a single ring closure product, tentatively identified as **30** (retention time 20.5 min); **30** could also be separated from **3** by silica gel chromatography. The chromatographic fractions richest in **26** were combined to give 155 mg of **26** [NMR 2.35 ppm (s, CH_3)] contaminated with 30% of **28** [NMR 2.50 ppm (s, CH_3)]. Preparation of **25** proceeded similarly, except that *o*-fluorobenzaldehyde (Aldrich Chemical Co.) was used.

4-Fluoro-*m*-toluic Acid (31). To a stirred solution of 56.7 mL of 2.5 M *n*-BuLi in 150 mL of Et_2O, cooled to −75 °C under N_2, was added dropwise 13.4 g (0.071 mol) of 3-bromo-4-fluorotoluene[25] in 50 mL of Et_2O. This was stirred in the cold for 3 h. The reaction mixture was then poured rapidly into a large beaker containing dry ice–Et_2O slush. This was loosely covered and stirred overnight. Approximately 400 mL of H_2O was added to the mixture, followed by 50 mL of 10% NaOH, and the two layers were separated. The aqueous layer was washed (Et_2O) and then acidified (4 N HCl). The resulting solid was collected by filtration, washed (H_2O), and dried to give 8.6 g (79%) of crude product, mp 154–158 °C (lit.[26] 159–160 °C).

2-Fluoro-5-methylbenzyl Alcohol (32). To a stirred solution of 50 mL (0.055 mol) of 1.1 M $LiAlH_4$ in 300 mL of Et_2O under N_2 was added dropwise, over a 1-h period, a solution of 8.0 g (0.05 mol) of 4-fluoro-*m*-toluic acid (**31**) in 150 mL of Et_2O. The mixture was refluxed for 4 h after the addition and stirring was continued for 2 h. Ethyl acetate was added dropwise to decompose excess $LiAlH_4$, followed by dilute H_2SO_4. The layers were separated and the Et_2O layer was washed with H_2O, saturated $NaHCO_3$, and again with H_2O, dried ($MgSO_4$), and concentrated. The crude product was chromatographed on silica gel with elution by $CHCl_3$. The first fraction was found to be the acetate of the desired product. This was hydrolyzed by refluxing for 2 h in 20% aqueous NaOH. The total product obtained was 6.7 g (92%) of 2-fluoro-5-methylbenzyl alcohol (**32**): IR (film) 3324 cm^{-1} (br, OH); MS *m/e* (rel intensity) 140 (100), 109 (99.8), 119 (85), 91 (70). This material was used directly in the preparation of **33**.

2-Fluoro-5-methylbenzyl Chloride (33). A solution of 6.1 g (0.043 mol) of 2-fluoro-5-methylbenzyl alcohol (**32**) and 25 mL of $SOCl_2$ was refluxed for 1.5 h. Excess $SOCl_2$ was distilled and then hexane was added to the residue. Concentration gave 5.8 g (85%) of **33**: MS *m/e* (rel intensity) 160 (6), 158 (17), 123 (100), 103 (22), 77 (22).

1-(2-Fluoro-5-methylphenyl)-2-(1-naphthyl)ethylene (34). 2-Fluoro-5-methylbenzyltriphenylphosphonium chloride was prepared by heating 2.6 g (0.017 mol) of **33** and 5.0 g (0.019 mol) of triphenylphosphine in 125 mL of dry benzene under reflux for 5 days. The product precipitated on cooling. The quaternary salt was collected by filtration, washed (cold benzene), and dried to give 1.6 g, mp 270–271.5 °C (23%).

To a mixture of 1.5 g (3.5 mmol) of the salt and 0.57 g (3.5 mmol) of 1-naphthaldehyde (Aldrich Chemical Co.) in 20 mL of methanol was added 4.8 mL of 1 M $LiOCH_3$. After stirring overnight, the solution was concentrated and the residue chromatographed on 100 g of silica gel with elution by 2:1 chloroform–hexane. This gave **34** (0.8 g, 87%) as an oil: MS *m/e* (rel intensity) 262 (100), 247 (71), 261 (46), 152 (37), 263 (20). Anal. ($C_{19}H_{15}F$) C, H, F.

2-(1-Naphthyl)propionic Acid (36). To a stirred solution of 51.3 g (0.4 mol) of naphthalene in 900 mL of dry THF under N_2 was added gradually 9.2 g (0.4 mol) of Na cut into small pieces. The resulting dark green mixture was stirred overnight. A solution of 32.6 g (0.175 mol) of 1-naphthylacetic acid (**35**, Aldrich Chemical Co.) in 200 mL of THF was added slowly to the mixture and the resulting dark red mixture was stirred overnight. A solution of 35.5 g (0.25 mol) of CH_3I in 100 mL of dry THF was then added dropwise, and the resulting colorless solution was stirred overnight.

H_2O (80 mL) was added followed by 90 mL of saturated Na_2CO_3. The layers were separated, and the aqueous layer was washed with ethyl acetate and then acidified with dilute HCl. This was extracted three times with ethyl acetate. The combined ethyl acetate extracts were washed (H_2O), dried ($MgSO_4$), and concentrated. The residue, which crystallized on standing, contained the desired acid as well as 1-naphthylacetic acid (**35**). The solid (15.8 g) was ground to a powder and washed (CCl_4) to remove **35**. The remaining solid was recrystallized from EtOH–H_2O to give colorless plates: mp 144–145.5 °C (8.0 g, 23%). Anal. ($C_{13}H_{12}O_2$) C, H.

2-(1-Naphthyl)propiophenone (37). Phenylmagnesium bromide was prepared from 2.37 g (0.015 mol) of bromobenzene and 0.36 g (0.015 mol) of Mg in 100 mL of ether. After refluxing the mixture for 1 h, it was cooled and then 1.65 g of dried $CdCl_2$ was added. The mixture was stirred for 2.5 h and then refluxed for 30 min. The ether was distilled and then 50 mL of dry benzene was added. This was partially distilled and then benzene was added to a total volume of 100 mL. To this was added dropwise a benzene (50 mL) solution of the acid chloride of **36**, prepared by refluxing 2.85 g of **36** and 20 mL of $SOCl_2$ and then distilling excess $SOCl_2$ in vacuo. The resulting mixture was refluxed for 4 h and then allowed to stir overnight. Ethyl acetate was added to the stirred mixture followed by saturated aqueous Na_2CO_3. The two layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined ethyl acetate solutions were washed (H_2O), dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel with elution by 3:1 hexane–CH_2Cl_2. The product was obtained as a viscous oil which crystallized on standing. This was recrystallized from 95% ethanol to give 1.0 g of colorless needles: mp 134.5–136 °C (25%); IR 1678 (s), 1592 cm^{-1} (m); MS m/e (rel intensity) 260 (50), 153 (72), 152 (49), 105 (100), 77 (90). Anal. ($C_{19}H_{16}O$) C, H.

1-Methoxy-2-(1-naphthyl)-1-phenylpropene (38). A mixture of 2.0 g (0.008 mol) of **37**, 4.9 g (0.046 mol) of trimethyl orthoformate, and a catalytic amount of *p*-toluenesulfonic acid in 50 mL of CH_3OH was refluxed for 4 days until TLC showed little starting material present. Dilute $NaOCH_3$ in CH_3OH was added dropwise to neutralize the solution. The solvent was evaporated in vacuo and the residue was heated to 230 °C for 4 h at 1.0 mm to remove CH_3OH, being careful not to distill the product. The material was then transferred to a smaller flask and distilled. The product was collected at 129–130 °C (0.1 mm) (0.97 g, 45.4%): MS m/e (rel intensity) 274 (100), 152 (76), 151 (73), 77 (73). Anal. ($C_{20}H_{18}O$) C, H.

Benzyl 1-Naphthyl Ketone. 1-Naphthylmagnesium bromide was prepared in the usual manner from 20.7 g (0.1 mol) of 1-bromonaphthalene (Aldrich Chemical Co.) and 2.5 g (0.1 mol) of Mg in 200 mL of Et_2O. After refluxing for 1 h, the mixture was cooled and 11.0 g (0.06 mol) of $CdCl_2$ was added. This was stirred (30 min) and then refluxed (3 h). The Et_2O was distilled and dry benzene added to a total volume of 200 mL; this was refluxed for 15 min. The mixture was cooled and a solution of 12.4 g (0.08 mol) of phenylacetyl chloride (Aldrich Chemical Co.) in 50 mL of benzene was added dropwise. This was stirred at room temperature (1 h) and then refluxed (1 h). After cooling, crushed ice was added followed by dilute H_2SO_4. The layers were separated and the benzene layer was washed with H_2O, then with saturated $NaHCO_3$, and again with H_2O, dried ($MgSO_4$), and concentrated. The residue crystallized and was recrystallized from 90% ethanol to give cream-colored plates: mp 65–66 °C (lit.[27] 55–77 °C as a mixture with benzyl 2-naphthyl ketone); 5.5 g (40%). Anal. ($C_{18}H_{14}O$) C, H.

2-Methoxy-2-(1-naphthyl)styrene. A mixture of 1.0 g (0.004 mol) of benzyl 1-naphthyl ketone, 1.0 mL of trimethyl orthoformate, and a catalytic amount *p*-toluenesulfonic acid in 30 mL of dry CH_3OH was refluxed overnight. The solution was filtered and then concentrated. The residue was heated to 170 °C at 1.0 mm of pressure for 2 h. The mixture obtained was chromatographed on silica gel with elution by 4:1 hexane–CH_2Cl_2. Two main fractions were isolated. The first fraction (A) solidified on standing and was recrystallized from 95% ethanol, mp 72–74 °C. The second fraction (B) was an oil, slightly contaminated with A. Both fractions gave 5-methoxychrysene (**12**) on irradiation by the usual method. Fraction A was converted to B when dissolved in benzene with a small amount of iodine and allowed to stand at room temperature. Thus A and B were the cis–trans isomers of 2-methoxy-2-(1-naphthyl)styrene. The isomers could be separated by GLC (column B 250 °C) (retention times, A 13.7 min, B 9.5 min); MS (isomer A) m/e (rel intensity) 260 (100), 229 (40), 217 (50), 215 (50), 202 (40), 167 (40); MS (isomer B) m/e (rel intensity) 260 (100), 229 (40), 217 (48), 215 (47), 202 (36), 167 (36); IR (isomer A) 1640 (s), 1592 (m), 1579 (w), 1569 cm^{-1} (w); IR (isomer B) 1637 (s), 1595 (m), 1578 (sh), 1572 cm^{-1} (w); NMR (isomer A) 3.40 ppm (3 H, s, CH_3); NMR (isomer B) 3.75 ppm (3 H, s, CH_3). Anal. ($C_{19}H_{16}O$, isomer A) C, H.

Photolysis of Alkenes to Chrysenes 2, 3, and 5–12. The procedure is illustrated with the synthesis of **6**. A solution of 800 mg (3.0 mmol) of 1-(4-fluorophenyl)-2-(1-naphthyl)propene (**19**) and 50 mg of iodine in 1 L of dry benzene was stirred and dry air was bubbled through the solution. This was irradiated with a Hanovia 250-W medium-pressure mercury lamp, using a corex filter. The reaction was monitored by GLC (column A, 250 °C). For 9-fluoro-5-methylchrysene (**6**), the reaction required 4.5 h. The mixture was filtered and the filtrate concentrated in vacuo. The residue was chromatographed on a silica gel column with 4:1 $CHCl_3$–hexane to give 220 mg of **6**. Further purification required chromatography on silica gel with cyclohexane and recrystallization from 95% ethanol to give colorless needles, 116 mg (13.1%), of greater than 99.9% purity according to analysis by TLC, GLC, and HPLC. Analytical data, melting points, yields, and UV spectra for **2–12** are given in Table I. Other spectral data are discussed below.

NMR and Mass Spectra of Methylchrysenes. In the NMR spectra of **1–9** and **11**, the methyl resonances were observed as singlets at 3.00–3.10 ppm; in **10** the methyl singlet appeared at 2.80 ppm. The methoxy derivatives **10** and **11** also gave singlets (OCH_3) at 3.65 and 4.12 ppm, respectively. The bay region hydrogens (H-4, H-10, H-11) of **1** resonated at 8.98 (H-4), 8.73, and 8.64 (H-10,11) and were the furthest downfield of the aromatic protons.[29] The aromatic protons of the modified chrysenes resonated between 6.95 and 8.90 ppm; only in **7** was a proton shifted further downfield (H-10, 9.15 ppm).

The mass spectra of the fluorinated 5-methylchrysenes were similar, showing the molecular ion (m/e 260) as base peak; M + 1, M − 1, M − 2, and M − 3 peaks were also observed as is typical for chrysenes. In addition, fragments of relative intensity 5–10% were observed at m/e 244, 239, and 233. The methoxy derivatives **10** and **11** showed principally M^+ 272 (100%), loss of 15 (20–60%), and 43 (50–85%).

α-Bromobenzyl 1-Naphthyl Ketone. To a stirred solution of 1.2 g (0.005 mol) of benzyl 1-naphthyl ketone in 30 mL of glacial HAc was added 3 drops of fuming HBr, followed by dropwise addition of 0.85 g (0.006 mol) of Br_2 in 15 mL of HAc. The mixture was stirred overnight and then poured into 200 mL of H_2O. The organic material was extracted with Et_2O and the Et_2O layer was carefully washed with 10% $NaHCO_3$ and then with H_2O, dried ($MgSO_4$), and concentrated. The oil obtained (1.3 g) was chromatographed on silica gel, eluting with 3:1 hexane–$CHCl_3$. This was used directly in the next step: IR (film) 1689 cm^{-1} (C=O); MS m/e (rel intensity) 324 (0.5), 215 (27), 202 (22), 155 (100), 127 (70), 126 (24).

α-Fluorobenzyl 1-Naphthyl Ketone. A mixture of 1.63 g (0.005 mol) of α-bromobenzyl 1-naphthyl ketone and 0.6 g (0.01 mol) of anhydrous KF in 20 mL of ethylene glycol was heated at 135 °C overnight with stirring. The mixture was cooled and then poured into 150 mL of H_2O. This was extracted with ethyl acetate. The ethyl acetate solution was washed (H_2O), dried ($MgSO_4$), and concentrated. The residue, 1.6 g of oil, was chromatographed on silica gel, eluting with 4:1 $CHCl_3$–hexane. The product was crystallized from ethanol–water to give 200 mg of cream-colored crystals: mp 69.5–70 °C; IR 1708 cm^{-1} (C=O); MS m/e (rel intensity) 264 (1), 156 (12), 155 (100), 127 (56), 126 (9). Anal. ($C_{18}H_{13}OF$) C, H, F.

5,6-Dihydro-5,6-dihydroxy-5-methylchrysene (39) and 11,12-Dihydro-11,12-dihydroxy-5-methylchrysene (40). A mixture of 1.0 g (0.004 mol) of 5-methylchrysene, 1.0 g of OsO_4, and 1.0 mL of pyridine in 80 mL of dry benzene was assembled under nitrogen and stirred at 60 °C for 40 days. The solvent was evaporated and 50 mL of CH_2Cl_2 was added, followed by 80 mL of 5 N NaOH and 25 mL of 1 M D-mannitol. This was stirred overnight. The two layers were separated and the CH_2Cl_2 layer

was washed with dilute HCl and then with H_2O, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel eluting with $CHCl_3$. The 5-methylchrysene eluted first (140 mg, 14%), followed by the 5,6-diol (600 mg, 54%) and finally the 11,12-diol (170 mg, 15%). Recrystallization of the 5,6-diol **39** from ethanol–water gave 548 mg of colorless prisms: mp 94.5–95 °C; IR 3400 cm^{-1} (br, –OH); MS *m/e* (rel intensity) 276 (18), 243 (30), 216 (25), 215 (100), 202 (19).

The 11,12-diol **40** (147 mg) was obtained as cream-colored plates from ethanol–water: mp 157.0–157.5 °C; IR 3300 cm^{-1} (br, –OH); MS *m/e* (rel intensity) 276 (48), 245 (59), 229 (73), 215 (100); NMR of **39** and **40**, see text. Anal. ($C_{19}H_{16}O_2$) C, H.

6-Fluoro-5-methylchrysene (4). A solution of 200 mg (0.7 mmol) of the 5,6-diol **39** in 25 mL of CH_2Cl_2 was added dropwise to a stirred solution of 180 mg (1.1 mmol) of freshly prepared diethylaminosulfur trifluoride in 25 mL of CH_2Cl_2 under nitrogen and cooled to –68 °C. The reaction was allowed to come to room temperature and stirred overnight. The reaction mixture was refluxed 30 min and cooled and then water was added. The methylene chloride layer was washed (H_2O), dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel with elution by 50:50 hexane–$CHCl_3$ to give **4** which was recrystallized from ethanol yielding 40 mg (22%) of colorless plates, mp 103.5–104 °C.

Reaction of 40 with DAST. A solution of 6.5 mg of **40** in 5 mL of CH_2Cl_2 was added dropwise to a stirred solution of 6 mg of DAST in 10 mL of CH_2Cl_2 cooled to –68 °C. The mixture was stirred at this temperature for 30 min and then allowed to warm to room temperature, stirring an additional 2 h. The mixture was concentrated and the residue was dissolved in 15 mL of dry benzene. A catalytic amount of *p*-toluenesulfonic acid was added and the mixture was refluxed for 4 h, cooled, and concentrated. The residue was chromatographed on silica gel, eluting with chloroform. The front running band was collected and analyzed by GLC–MS (column A, 250 °C). Only 11-fluoro-5-methylchrysene (**7**) (retention time 24.5 min) was observed; 12-fluoro-5-methylchrysene (**8**) (retention time 25.6 min) was not detected.

Assays for Mutagenicity. *S. typhimurium* TA-100 was obtained from Dr. B. N. Ames and assays were carried out as previously described.[28] For enzymatic activation of the chrysenes, the S-9 fraction was obtained from the livers of male F-344 rats (250–300 g) which had been treated 5 days prior to sacrifice with 500 mg/kg of Arochlor 1254 (Analabs, Inc.). The optimum ratio of the S-9 fraction (33 μg of protein/μL) to 5-methylchrysene (in 10 μL of Me_2SO) per plate was determined to be 200 μL of S-9/20 μg of 5-methylchrysene. A dose–response curve for 5-methylchrysene was constructed; maximum mutagenicity was obtained for 20 μg of **1**/plate. The modified chrysenes were all tested at doses of 20 μg/plate (in 10 μL of Me_2SO). Qualitative comparisons of mutagenic activity were based on these single doses. Each assay was run in duplicate and three separate assays were performed for each compound; thus the values reported (Table II) are averages of six determinations. Survivor studies using 20 or 50 μg of the appropriate chrysene did not indicate any significant bactericidal or bacteriostatic effect on *S. typhimurium* TA-100, except for 7-fluoro-5-methylchrysene.

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