Compound **5** produced a variable effect on hepatic triglyceride content since low doses (10 mg/kg) reduced but higher doses (50 and 100 mg/kg) elevated liver triglyceride levels (Table V). The high doses (50 and 100 mg/kg) of compound **5** did not lower hepatic triglyceride biosynthesis measured in vivo more than a 10 mg/kg dose (Table IV). Therefore, the significantly lower serum triglyceride concentration associated with the 50 mg/kg dose of compound **5** compared to the 10 mg/kg dose (Table V) is probably the result of a decrease in hepatic triglyceride (very low density lipoprotein, VLDL) release since liver triglyceride content increased significantly. None of the doses (10–100 mg/kg) of compoumd **5** used in these studies killed any animals or produced abnormal alterations in body weight gain and food intake. Therefore, inhibitors of hepatic phosphatidate phosphohydrolase activity may effectively reduce hepatic triglyceride biosynthesis in vivo and lower serum triglyceride (VLDL) levels.

The mechanism(s) by which compound **5** might lower serum cholesterol have not been studied directly. However, the observation that compound **5** reduces serum triglyceride content (7 days) before altering serum cholesterol levels (16 days) might indicate that these responses are interrelated. This seems reasonable since it is well established that serum VLDL (triglyceride rich) is the precursor of serum LDL (low density lipoprotein, cholesterol rich).[14] Therefore, reductions in the production and secretion of hepatic triglyceride (VLDL) should lower serum cholesterol (LDL), provided reductions in the clearance of these lipoproteins do not occur.

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Synthesis and Carcinogenic Activity of 5-Fluoro-7-(oxygenated methyl)-12-methylbenz[*a*]anthracenes

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Treatment of 7,12-benz[*a*]anthraquinone (**2**) with methylmagnesium iodide or methyllithium yields mixtures of *cis*- and *trans*-7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracenes (**3a,b**), in which the ratio of cis to trans lies in the 3–4:1 region. Each isomer afforded high yields of 7-chloromethyl-12-methylbenz[*a*]anthracene (**5**) on treatment with hydrogen chloride in ethyl acetate. Similarly, 5-fluoro-7,12-benz[*a*]anthraquinone (**8**) afforded a mixture of *cis*- and *trans*-5-fluoro-7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracenes (**9**) which yielded 7-chloromethyl-5-fluoro-12-methylbenz[*a*]anthracene (**10**) on treatment with HCl. The chloromethyl compounds, **5** and **10**, yielded 7-acetoxymethyl-12-methylbenz[*a*]anthracene (**6**) and 7-acetoxymethyl-5-fluoro-12-methylbenz[*a*]anthracene (**11**) on treatment with acetate ion. Hydrolysis of **6** and **11** yielded 7-hydroxymethyl-12-methylbenz[*a*]anthracene (**7**) and 5-fluoro-7-hydroxymethyl-12-methylbenz[*a*]anthracene (**12**), respectively. Since neither **11** nor **12** is appreciably carcinogenic, the carcinogenic metabolism of 7,12-dimethylbenz[*a*]anthracene (DMBA) probably does not involve attack at the 7-methyl group.

One of the chief methods for preparing 7,12-dimethylbenz[*a*]anthracene (DMBA, **1**), a compound of widespread interest in cancer research, involves the reaction of 7,12-benz[*a*]anthraquinone (**2**) with methylmagnesium iodide to form 7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracene (**3**) which is converted into 7-iodomethyl-12-methylbenz[*a*]anthracene (**4**) by treatment with methanolic HI.[2] Reduction of **4** yields **1**.

Because of instability **4** is not a good intermediate if oxygenated substitution products on the 7-methyl group are desired. As we were interested in preparing such oxygenated derivatives not only of **1** but also of 5-fluoro-7,12-dimethylbenz[*a*]anthracene[3] (**7**), we have reinvestigated the above synthetic route. The oxygenated derivatives of **7** were desired for testing as to carcinogenic activity because of the hypotheses that the metabolic pathway important in the carcinogenic metabolism of all 7-methylbenz[*a*]anthracenes may involve oxygenation or other reactions at the 7-methyl groups.[4]

The reactions of **2** with methylmagnesium bromide and iodide or methyllithium are difficult to reproduce. In all cases varying amounts of unreacted quinone are present regardless of the excess organometallic reagent used or the time of reaction. We believe these results can be explained by the formation of a complex between quinone and the salt of the diol produced. This complex evidently resists further reaction with excess organometallic reagent but we do not understand why the amount of this complex varies so widely in different experiments.

Only one isomer, mp 181.5–182.5 °C, has been reported from the reaction of **2** with methylmagnesium iodide.[5] This was shown to be the cis isomer by comparison[6] with

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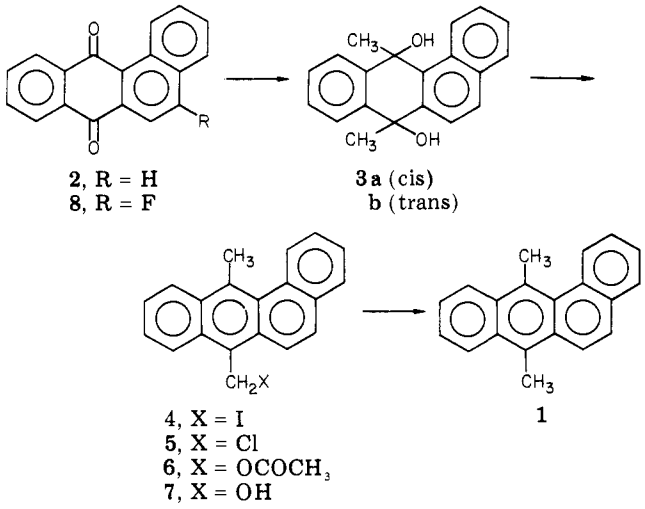
Scheme I



2, R = H
8, R = F

3a (cis)
b (trans)

4, X = I
5, X = Cl
6, X = $OCOCH_3$
7, X = OH

1

the diol formed by catalytic reduction of the 7,12-peroxide[7] of **1**. We have confirmed this finding with diol produced by reduction of the peroxide with $LiAlH_4$. We have also isolated the *trans*-diol **3b**, the minor component,[8] by chromatography of the crude diol mixtures formed by reaction of methyl organometallic derivatives with **2**. The ratio of *cis*- to *trans*-diol is little affected by the organometallic reagent used.

For further transformation of the diols treatment of an ethyl acetate solution with dry HCl afforded 7-chloromethyl-12-methylbenz[*a*]anthracene (**5**) in high yield.[9] The stability of **5** is excellent and it was readily reduced to **1** or reacted with acetate ion to produce 7-acetoxymethyl-12-methylbenz[*a*]anthracene (**6**).

In a similar reaction 5-fluoro-7,12-benz[*a*]anthraquinone (**8**) has been converted to a mixture of *cis*- and *trans*-5-fluoro-7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracene (**9**) which readily yielded 7-chloromethyl-5-fluoro-12-methylbenz[*a*]anthracene (**10**) on treatment with HCl. Reaction with acetate yielded 7-acetoxymethyl-5-fluoro-12-methylbenz[*a*]anthracene (**11**) from which 5-fluoro-7-hydroxymethyl-12-methylbenz[*a*]anthracene (**12**) was obtained on hydrolysis. Reduction of **10** yielded 5-fluoro-7,12-dimethylbenz[*a*]anthracene (**13**). This new synthesis of **13** is preferable to the older one.[3]

Compounds **11** and **12** did not produce tumors in 17 months in male rats after subcutaneous injection.[10a] Slight activity for **11** was noted[10b] but none for **12**.[10b] We are at present preparing the 9-fluoro analogues of **11** and **12** for testing.

These findings are of interest with regard to hypotheses as to why 7-methylbenz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene are such potent carcinogens as compared to benz[*a*]anthracene and 12-methylbenz[*a*]anthracene. One hypothesis (see discussions in ref 4) has it that metabolism leading to cancer takes place on the 7-methyl group. Our thoughts are that the 7-methyl group merely serves to block a detoxification metabolism which is initiated at the 7 position. When the detoxification pathway is blocked the cancer-producing metabolism can become effective.[11] What positions are important for the carcinogenic metabolism in 7,12-DMBA is yet to be determined. Results at present indicate that 1-fluoro-,[12] 2-fluoro-,[12,13] and 5-fluoro-DMBA[13] are inactive; hence, one can assume that substitution in any of the 1, 2, and 5 positions is sufficient to block the carcinogenic pathway. Significantly 1,7,12-, 2,7,12-, and 5,7,12-trimethylbenz[*a*]anthracenes are also noncarcinogenic.[13] Since **11** and **12** are essentially inactive one can argue that metabolism at the 7-methyl group in 5-fluoro-7,12-DMBA is not effective in inducing cancer.

Experimental Section

All melting points are uncorrected. The term "worked up as usual" means that an ether–benzene solution of the reaction products was washed with dilute acid and/or alkali, with saturated salt solution, and was filtered through a cone of $MgSO_4$. The solvent was then removed on a rotary evaporator and the residue treated as indicated. All new compounds marked with an asterisk gave acceptable analyses (±0.3%) as reported by M-H-W Laboratories, Garden City, Mich. Satisfactory NMR, IR, and mass spectra were obtained for all new compounds.

cis*- and *trans*-7,12-Dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracene (3a,b).** In general, solutions of **2** in benzene–ether were added gradually to solutions of excess methylmagnesium iodide or bromide or methyllithium in ether. Refluxing was varied from 6 to 20 h. After cooling ammonium chloride solution was added and the reaction products were isolated in a conventional way from solutions which had been dried by passing through anhydrous $MgSO_4$. The diol mixtures, obtained in 60–90% yield, were purified by recrystallization if TLC showed only a small amount of **2**. In general, smaller amounts of **2** were present when CH_3Li was used. If larger amounts of **2** were present chromatography over alumina was used. The proportion of **3a** to **3b** lay in the 3–4 to 1 ratio. The order of elution from Al_2O_3 using benzene and then benzene–$CHCl_3$ mixtures was **2**, **3b**, **3a**. Pure **3a** melted at 180.3–182.0 °C and pure **3b at 156.5–158.0 °C, but the melting point of each was affected by small amounts of the solvent of recrystallization. The analytical samples had no solvent and were obtained by crystallization from benzene–petroleum ether: bp 65–110 °C; NMR [$CDCl_3$, $(CH_3)_4Si$] for **3a** δ 1.45 (s, 3, CH_3), 1.78 (s, 3, CH_3), 2.42 (s, 2, OH), 7.15–7.80 (m, 9, arom), 9.00–9.33 (m, 1, arom); NMR for **3b** δ 1.77 (s, 3, CH_3), 1.97 (s, 3, CH_3), 2.36 (br s, 1, OH), 2.57 (br s, 1, OH), 7.17–7.92 (m, 9, arom), 9.00–9.43 (m, 1, arom).

7-Chloromethyl-12-methylbenz[*a*]anthracene (5). The synthesis of **5** from **3a** has been described.[9] The purest sample of **5** melted at 142.5–142.9 °C.[14] When HCl gas was passed into a solution of 0.20 g of pure **3b** in 7 mL of ethyl acetate at 0 °C to saturation the solid which separated, 0.16 g (80%), melted at 142.5–142.9 °C alone and mixed with the above sample obtained from **3a**: NMR δ 3.18 (s, 3, CH_3), 5.38 (s, 2, CH_2Cl), 7.10–8.45 (m, 10, arom).

5-Fluoro-7,12-benz[*a*]anthraquinone* (8). A mixture of 40 g of 3-(4-fluoro-1-naphthyl)phthalide,[15] 750 mL of acetic acid, and 200 g of activated ($CuSO_4$) zinc dust was stirred at reflux for 24 h. After cooling and standing the clear acetic acid solution was poured into water. Acidification of an alkaline solution of the reaction product afforded an almost quantitative yield of 2-(4-fluoro-1-naphthylmethyl)benzoic acid,[16] mp 173–175 °C. After keeping a solution of 24 g of this acid in 150 mL of concentrated H_2SO_4 at room temperature for 2 h, the dark orange-red solution was poured on ice. There was obtained an almost quantitative yield of crude 5-fluoro-7,12-dihydro-7-oxobenz[*a*]anthracene[16] which melted in the 170–176 °C range. On recrystallization of a portion from benzene–ethanol a form, mp 277–279 °C, was obtained which had a strong carbonyl absorption and very little hydoxyl in the IR. Undoubtedly a conversion from anthrol to anthrone had occurred. The crude product was heated at reflux for 1.5 h with a solution of 33 g of $Na_2Cr_2O_7 \cdot 2H_2O$ and 150 mL of acetic acid. On pouring into water there was isolated crude quinone **8** in 84% yield. A sample recrystallized from benzene–ethanol melted at 161–162 °C.

5-Fluoro-7,12-dihydro-7,12-dihydroxy-7,12-dimethylbenz[*a*]anthracene* (9). In the best of many experiments a solution of 5.5 g of **8** in 300 mL of 1:2 ether–benzene was added to the methylmagnesium bromide prepared from 2.4 g of sublimed magnesium in 200 mL of ether. After being held at reflux for 1.5 h the mixture was treated with aqueous NH_4Cl and worked up as usual to yield a residue which was triturated with $CHCl_3$. The insoluble diol, melting point in the 200–209 °C range, was assumed to be the cis form in analogy with the results in the case of **3a**. The pure isomer, mp 209–210 °C, was obtained by recrystallization from benzene–ethanol. The isomeric diol *trans*-**9** was never obtained in analytically pure condition because of difficulty of

Table I

no. of rats	DMBA derivatives	rats with tumor
15	5-F-7-OAc-DMBA	3/13[a]
21	5-F-7-OH-DMBA	0/14
21	5-F-DMBA	1/12[b]
11	control	0/8

[a] Tumor induction time (days), mean ± SE = 205.7 ± 18.2; range = 173-236. [b] Tumor induction time (days), mean ± SE = 231; range = 231.

removing all solvent from the crystals which melted in the 98–100 °C range with decomposition. A representative sample of reaction products was separated by column chromatography over alumina, eluting with benzene and benzene–$CHCl_3$, to yield 59% of *cis*-**9**, 26% of *trans*-**9**, and 7% of **8**. Similarly when CH_3Li in ether (0.1 mol, Alpha Products) was added to a solution of 5.5 g of **8** in 150 mL of ether and 350 mL of benzene there was obtained 87% of *cis*-**9** and *trans*-**9** (isolated yields 61 and 26%, respectively) and no **8**. The best results seemed to be obtained with both Grignard and lithium reagents when the ratio of benzene to ether was higher than 1:1.

7-Chloromethyl-5-fluoro-12-methylbenz[*a*]anthracene* (**10**). In a typical reaction dry HCl was passed into an ice-cooled suspension of 1.00 g of *cis*-**9** in 20 mL of pure ethyl acetate. After 10–20 min a clear solution results (sooner in larger runs because the exothermic reaction often raised the temperature to 35–40 °C). After standing at 0–5 °C for 1 h the solid, mp 130–138 °C, was collected in 95% yield. Recrystallization from ether–petroleum ether afforded pure **10**, mp 139–140 °C.

7-Acetoxymethyl-5-fluoro-12-methylbenz[*a*]anthracene* (**11**). A solution of 2 g of dry KOAc, 50 mg of 18-crown-6 ether (Aldrich), and 3.1 g of **10** in 60 mL of THF was held at reflux for 20 h. After the usual workup there was obtained 2.8 g (84%) of **11**, mp 109–110.5 °C. One recrystallization from benzene–hexane raised the melting point to 112.0–112.5 °C with little loss. A similar result was obtained by refluxing a stirred mixture of the same components in benzene for 5 h.

5-Fluoro-7-hydroxymethyl-12-methylbenz[*a*]anthracene* (**12**). A mixture of 3.95 g of **11**, 10 mL of methanol, and 20 mL of 10% NaOH was refluxed for 2 h. After the usual workup there was obtained 3.15 g (91%) of **12**, mp 142–143 °C, after one recrystallization from benzene–hexane.

A. Long-Term Subcutaneous Tests of DMBA, 11, and 12 on Male Fischer Rats.[10a] Groups of 18 male Fischer rats (Charles River Breeding Laboratory, Wilmington, Mass.) were injected subcutaneously in the right hind leg once with 2.0 mg of 7,12-dimethylbenz[*a*]anthracene or four times at weekly intervals with 2.3 mg of 5-fluoro-7-hydroxymethyl-12-methylbenz[*a*]anthracene, 2.7 mg of 5-fluoro-7-acetoxymethyl-12-methylbenz[*a*]anthracene, or only the solvent. Each dose was dissolved with mild heat in 0.25 mL of sterile trioctanoin (Sigma Chemical Co.). The rats were maintained in individual wire-mesh cages and were fed Wayne Breeder Blox (Allied Mills, Inc., Chicago, Ill.). Of the 18 rats that received a single injection of 7,12-dimethylbenz[*a*]-anthracene, 6, 15, and 17 had sarcomas at the injection site by 6, 9, and 16 months, respectively. By contrast, 1, 0, and 0, respectively, of the rats that received four injections of 5-fluoro-7-hydroxymethyl-12-methylbenz[*a*]anthracene, 5-fluoro-7-acetoxymethyl-12-methylbenz[*a*]anthracene, or the solvent alone developed sarcomas by the termination of the experiment at 16 months. All of the rats injected with the fluorinated hydrocarbons and all but one of those that received only injections of the solvent survived to the end of the experiment. All of the rats were subjected to routine gross autopsies of the skin and subcutaneous tissues and of the organs of the abdominal and thoracic cavities. The only gross pathology observed, other than the sarcomas indicated above, was the presence of interstitial cell tumors of the testes. The latter tumors occur spontaneously in old Fischer rats, and the incidence of these tumors was not increased by the administration of the hydrocarbons.

B. Sarcoma at Site of Injection in Sprague–Dawley Male Rats Caused by 8, 11, and 12.[10b] A 0.1-μmol dose of the test compound was given by sc injection in 0.1 mL of sesame oil three times a week for 30 doses beginning at 30 days of age. The observation period was 270 days.

Male rats were purchased from Sprague–Dawley Farms, Inc., Madison, Wis., and housed in wire cages in a constant temperature animal room with an alternating light–dark cycle of 12 h. Rats bearing tumors were killed 30 days after the appearance of the first palpable tumor. The remainder were sacrificed after 270 days of observation (see Table I).

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