Preparation of Hydroxy Derivatives of 7,12-Dimethylbenz[*a*]anthracene: 8-Hydroxy-7,12-dimethylbenz[*a*]anthracene, 10-Hydroxy-7,12-dimethylbenz[*a*]anthracene, and 11-Hydroxy-7,12-dimethylbenz[*a*]anthracene

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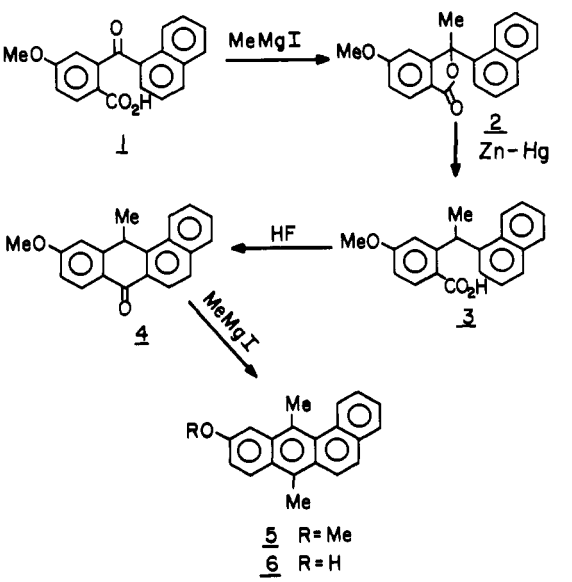
The synthesis of hydroxy derivatives of 7,12-dimethylbenz[*a*]anthracene (DMBA) at positions 8, 10, and 11 is described. 2-(1-Naphthoyl)-4-methoxybenzoic acid (1) was prepared from 4-methoxyphthalic anhydride by Grignard synthesis. Similarly 2-(1-naphthoyl)-6-methoxybenzoic acid (7) and 2-(1-naphthoyl)-3-methoxybenzoic acid (7a) were prepared from 3-methoxyphthalic anhydride. The acids 1, 7, and 7a gave the lactones 3-methyl-3-(1-naphthyl)-5-methoxyphthalide (2), 3-methyl-3-(1-naphthyl)-7-methoxyphthalide (8), and 3-methyl-3-(1-naphthyl)-4-methoxyphthalide (8a), respectively, on treatment with methylmagnesium iodide. Reduction of 2 under Clemmensen conditions gave 2-(α-1-naphthylethyl)-4-methoxybenzoic acid (3). Reduction of 6 and 8a with Zn–Cu gave 2-(α-1-naphthylethyl)-6-methoxybenzoic acid (9) and 2-(α-1-naphthylethyl)-3-methoxybenzoic acid (9a), respectively. Cyclization of the acids 3, 9, and 9a in HF gave the tetracyclic ketones 10-methoxy-12-methyl-7(12*H*)-benz[*a*]anthracenone (4), 8-methoxy-12-methyl-7(12*H*)-benz[*a*]anthracenone (10), and 11-methoxy-12-methyl-7(12*H*)-benz[*a*]anthracenone (10a), respectively. Reaction of these ketones with methylmagnesium iodide gave 10-methoxy-DMBA (5), 8-methoxy-DMBA (11), and 11-methoxy-DMBA (11a), which were cleaved by BBr_3 to 10-hydroxy-DMBA (6), 8-hydroxy-DMBA (12), and 11-hydroxy-DMBA (12a). These hydroxy derivatives are being used as primary standards in the identification of metabolites of DMBA in animal studies.

Studies designed to describe the mechanism by which polycyclic aromatic hydrocarbons cause biological changes rely on the ability to identify metabolic products of the parent hydrocarbons. The powerful mammary carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) is metabolized by rat liver to numerous products, most of which remain unidentified due to the lack of authentic standards for comparison purposes (*12*). Prominent metabolic reaction has been speculated to occur in the ring with positions 8, 9, 10, and 11 (*2*), but the only known phenols of DMBA are DMBA-4-ol (*3*) and DMBA-5-ol (*6, 8*).

One avenue leading to the benz[*a*]anthracene ring system is that which employs the reaction of phthalic anhydrides with naphthylmagnesium halides. By this route, Newman prepared a number of methyl substituted benz[*a*]anthracene derivatives (*7*), but methoxyphthalic anhydrides, which would lead to hydroxy substituted benz[*a*]anthracenes, have not been thoroughly explored in this type of reaction sequence.

The reaction of 4-methoxyphthalic anhydride with 1-naphthylmagnesium bromide has been reported to give 2-(1-naphthoyl)-4-methoxybenzoic acid (*13*), the structure of which was verified by decarboxylation to 1-naphthyl-3-methoxyphenyl ketone (*4*). The keto acid **1** (Chart I) reacted with methyl magnesium iodide to give the expected lactone **2** which was reduced under Clemmensen conditions to the benzoic acid derivative **3**.

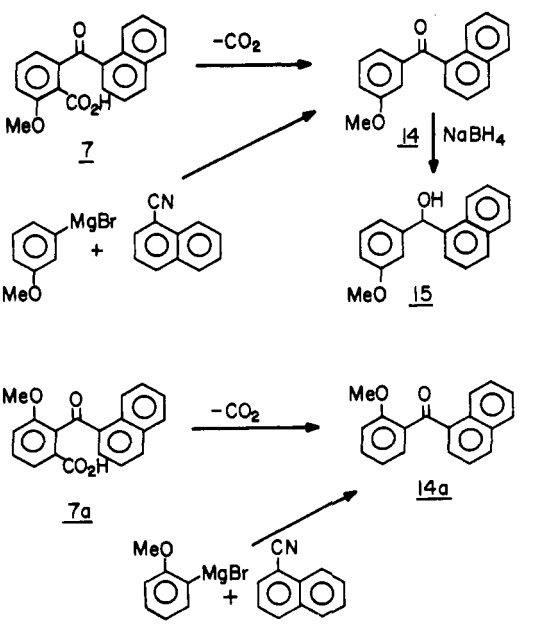
Chart I



The acid **3** easily cyclized in hydrogen fluoride to give the tetracyclic ketone **4**. Introduction of the 7-methyl group with methylmagnesium iodide followed by dehydration gave the expected methoxybenzanthracene **5** which was cleaved with boron tribromide to give DMBA-10-ol **6**.

In a fashion similar to that described for the acquisition of **1**, the isomeric compounds 2-(1-naphthoyl)-6-methoxybenzoic acid (**7**) and 2-(1-naphthoyl)-3-methoxybenzoic acid (**7a**) were pre-

Chart II



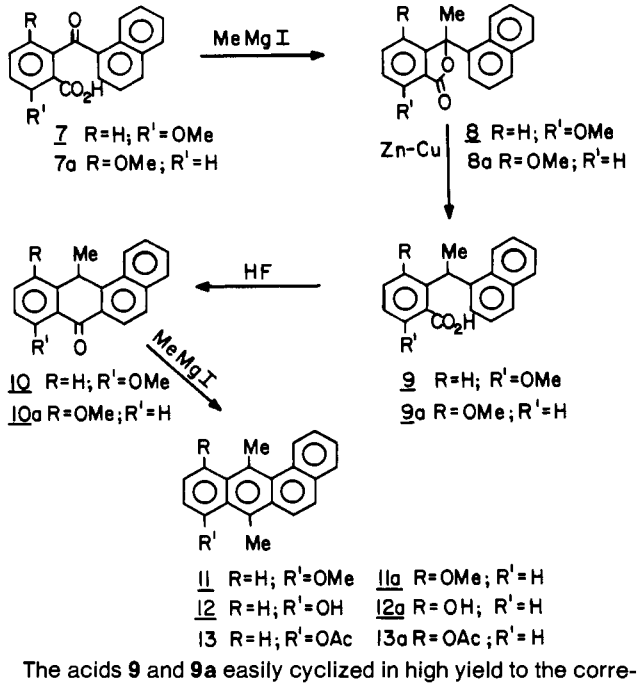
pared by the reaction of 3-methoxyphthalic anhydride with 1-naphthylmagnesium bromide. The identification of these two acids was performed by decarboxylation of **7** to *p*-methoxyphenyl 1-naphthyl ketone **14** and of **7a** to *o*-methoxyphenyl naphthyl ketone **14a** (Chart II) in a fashion similar to that described by Rapoport for the degradation of phenanthroic acids (*11*).

The Grignard method of Newman (*9*) was applied for the independent acquisition of ketones **14** and **14a** from the reaction of 1-cyanonaphthalene with *m*-methoxyphenylmagnesium bromide and *o*-methoxyphenylmagnesium bromide, respectively. Identification of the individual ketones was achieved by thin-layer chromatography (TLC) on silica gel, and comparison of infrared spectra.

The identity of **14a** was further established by melting point and mixture melting point. Compound **14,** being a liquid, was further characterized by sodium borohydride reduction of both the sample obtained by decarboxylation and the sample obtained by the alternate Grignard synthesis. Both procedures gave the solid carbinol **15** which was characterized by TLC, melting point and mixture melting point, and infrared spectra.

Reaction of compound **7** with methylmagnesium iodide gave the lactone **8,** but reduction of this lactone under Clemmensen conditions, as outlined for compound **2,** gave exclusively decarboxylation products. Also, lactone **8a,** formed from **7a** by the Grignard procedure, gave only decarboxylation products. Both **8** and **8a** were successfully converted into the corresponding acids **9** and **9a** by reduction in sodium hydroxide solution with a zinc–copper alloy (Chart III).

Chart III



The acids **9** and **9a** easily cyclized in high yield to the corresponding tetracyclic ketones **10** and **10a** in hydrogen fluoride. Both **10** and **10a** gave the fully aromatic methoxy-7,12-dimethylbenz[*a*]anthracene derivatives **11** and **11a** upon treatment with methylmagnesium iodide followed by mild acid dehydration.

The phenols DMBA-8-ol, **12,** and DMBA-11-ol, **12a,** were obtained by cleavage of the ethers with boron tribromide. The light yellow solutions darkened quickly and the individual phenols were identified by conversion to the acetates **13** and **13a.** Although the phenols were not obtainable in crystalline form, they gave single spots on TLC for 1 week when stored in ethanol at 5 °C. Also, conversion to the heptafluorobutyrate esters and gas chromatographic (GC) analysis by electron capture detection showed no diminution in the peak height over a 1-week period. However, in 2 weeks, definite degradation was noticeable by GC.

Metabolic studies thus far with rat liver do not reveal the presence of monohydroxy derivatives of DMBA. However, the availability of the monohydroxy compounds reported here has made possible the identification of 8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz[*a*]anthracene as a key metabolite of DMBA by rat liver microsomes since it forms DMBA-8-ol in the presence of mild acids (*5*).

Experimental Section

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The IR spectra of all compounds were consistent with the proposed structures. Elemental analyses (C, H) within ±0.4% of the theoretical values were submitted for review and were performed by Atlantic Microlab, Atlanta, Ga.

***3-Methyl-3-(1-naphthyl)-5-methoxyphthalide* (*2*).** A solution of 36.6 mmol of methylmagnesium iodide in 300 ml of ether was added dropwise to a solution of 10.2 g (33.3 mmol) of 2-(1-naphthoyl)-4-methoxybenzoic acid (**1**) in 300 ml of hot benzene. After refluxing for 2 h the solution was allowed to stand at room temperature (24 °C) for 16 h. The suspension was acidified with cold, dilute HCl to pH 1 and extracted with chloroform. Following drying with Na_2SO_4 and evaporation, the residue was recrystallized to give 6.0 g (59.2%) of light pink needles, mp 206–207 °C. Anal. ($C_{20}H_{16}O_3$) C, H.

***2-[α-1'-(Naphthyl)ethyl]-4-methoxybenzoic Acid* (*3*).** A solution of 4.75 g (15.1 mmol) of the lactone **2** in 60 ml of water, 120 g of Zn–Hg, 120 ml of toluene, 225 ml of acetic acid, and 90 ml of concentrated HCl was refluxed for 2 h, diluted with 1 l. of water, and extracted with three 500-ml portions of ethyl acetate. The organic layer was then extracted with five 200-ml portions of 5% K_2CO_3. Acidification of the aqueous phase with cold, dilute HCl gave a precipitate which, when recrystallized from ethyl acetate, gave 3.0 g (62.7%) of the acid **3,** mp 220–221 °C. Anal. ($C_{20}H_{18}O_3$) C, H.

***10-Methoxy-12-methyl-7(12H)-benz[a]anthracenone* (*4*).** After standing for 30 min, a solution of 2.4 g (7.84 mmol) of the acid **3** in 35 ml of anhydrous HF was poured into 200 ml of water and 100 g of ice. The emulsion was extracted with two 500-ml portions of chloroform. The chloroform was washed with two 400-ml portions of water, dried with Na_2SO_4 and evaporated to yield an oil which crystallized. Recrystallization from 10 ml of benzene and 6 ml of heptane gave 1.78 g (78.7%) of the anthrone **4** mp 123–124 °C. Anal. ($C_{20}H_{16}O_2$) C, H.

***10-Methoxy-7,12-dimethylbenz[a]anthracene* (*5*).** An ether solution of methylmagnesium iodide (5.56 mmol) was added dropwise to a solution of 1.60 g (5.56 mmol) of the anthrone **4** in 20 ml of ether and 50 ml of benzene and the mixture was stirred for 16 h. The complex was decomposed with cold, dilute HCl and extracted with benzene. The ether–benzene solution was evaporated to give a yellow solid to which was added 500 mg of *p*-toluenesulfonic acid monohydrate in 100 ml of benzene. The solution was refluxed for 1 h, washed with two 100-ml portions of water, dried with Na_2SO_4, evaporated, and crystallized from benzene–heptane to give 1.16 g (73.4%) of light yellow-green fluorescent crystals, mp 137.5–138.5 °C. Anal. ($C_{21}H_{18}O$) C, H.

***10-Hydroxy-7,12-dimethylbenz[a]anthracene* (*6*).** A solution of 3 ml of BBr_3 in 10 ml of benzene was added dropwise to a solution of 200 mg (0.74 mmol) of methoxy compound **5** in 30 ml of benzene. After refluxing for 1 h, the solution was poured into a solution of 100 ml of water and 20 g of ice. The aqueous phase was extracted with ethyl acetate, and the combined organic layers were washed with three 50-ml portions of water, dried with Na_2SO_4, and evaporated to give a dark brown oil. The crude phenol was applied to a 10 cm × 2 cm silica column and eluted with benzene to give a solid which was recrystallized from

cyclohexane to give 120 mg (63.2%) of the phenol **6** as light tan crystals, mp 122–123 °C. Anal. ($C_{20}H_{16}O$) C, H.

3-Methoxyphthalic Acid. A mixture of 50 g (0.367 mol) of 2,3-dimethylanisole and 348 g (2.2 mol) of $KMnO_4$ in 3 l. of water was combined in a 5-l. three-necked flask fitted with two condensers. The mechanically stirred mixture was gradually heated on a steam bath until a vigorous reaction ensued, which was partially subdued by the application of ice-cold wet towels. Refluxing was continued for 1.25 h until the purple color of permanganate had dissipated. The mixture was then filtered, and the MnO_2 precipitate washed with water. The filtrate was cooled in an ice bath, acidified with concentrated HCl, saturated with NaCl, and extracted with ether. The ethereal extract was washed with saturated NaCl solution, dried ($MgSO_4$), and concentrated in vacuo to give 33.8 g (46.8%) of crude 3-methoxyphthalic acid as a white solid. Continuous ether extraction of the aqueous phase yielded an additional 37 g of crude diacid, for a total of 70.8 g (97.3%) of crude product. This was recrystallized from ethyl acetate–benzene and gave 40.4 g of crystalline product in the first crop, mp 174–176 °C (lit. (*1*) mp 173–174 °C).

Subsequently it was discovered that use of pyridine as a cosolvent greatly facilitated the handling of this reaction during the $KMnO_4$ addition phase and gave a much higher yield of product in the first crop. Thus, when 80 g (0.587 mol) of 2,3-dimethylanisole was oxidized in 1200 ml of water and 600 ml of pyridine with the $KMnO_4$ (557 g) being added in 10-g aliquots over a period of 2 h, a first crop yield of 86.6 g (75.3%) of solid diacid was obtained. The latter method was used for preparative purposes.

2-(1-Naphthoyl)-6-methoxybenzoic Acid (7) and 2-(1-naphthoyl)-3-methoxybenzoic Acid (7a). A mixture of 31 g (0.158 mol) of 3-methoxyphthalic acid and 100 ml of acetyl chloride in 30 ml of anhydrous *p*-dioxane was refluxed for 1.5 h. The solution was then concentrated in vacuo using benzene to azeotropically remove the last traces of acetic acid. The crude solid 3-methoxyphthalic anhydride was refluxed in 100 ml of anhydrous benzene, and to it was added the ether solution of 1-naphthylmagnesium bromide obtained from 34.4 g (0.166 mol) of 1-bromonaphthalene and 4.04 g (0.166 mol) of magnesium. The resultant mixture was refluxed for 2 h and left to stir overnight at room temperature.

The yellow suspension was poured onto a mixture of 10% HCl and ice, extracted with ether, and washed with water. The organic solution was then extracted with 5% NaOH, the latter extract was cooled in an ice bath, acidified with concentrated HCl, extracted with ethyl acetate, washed with water, dried ($MgSO_4$), and concentrated in vacuo to give 42.9 g (88.4%) of a crude mixture of the two isomeric naphthoylbenzoic acids **7** and **7a** as a dark yellow glass. This mixture was chromatographed on a 50 × 800 mm silica gel (80–200 mesh) column using benzene to pack the column and increasing concentrations of ethyl acetate in benzene to elute the first isomer, which proved to be 2-(1-naphthoyl)-3-methoxybenzoic acid (**7a**), in 14.9 g (34.8%) yield followed by 4.0 g of a mixed fraction. The more polar 2-(1-naphthoyl)-6-methoxybenzoic acid (**7**) was eluted with 2% acetic acid in ethyl acetate, and was obtained in 6.1 g (14.2%) yield. Analytical samples of the two isomers were obtained by crystallization from benzene.

2-(1-Naphthoyl)-3-methoxybenzoic acid (**7a**), mp 197–198 °C. Anal. ($C_{19}H_{14}O_4 \cdot \frac{1}{4}H_2O$) C, H; and 2-(1-naphthoyl)-6-methoxybenzoic acid (**7**), mp 200–201 °C. Anal. ($C_{19}H_{14}O_4 \cdot \frac{1}{4}H_2O$) C, H.

Structure Proof of 2-(1-Naphthoyl)-6-methoxybenzoic Acid (7). A mixture of 1.1 g (3.58 mmol) of ketoacid **7** and 100 mg of $CuSO_4$ in 20 ml of quinoline was refluxed for 3 h, cooled to room temperature and poured onto crushed ice/10% HCl, and extracted with ether. The organic solution was extracted with 10% HCl to remove the quinoline, washed with water, saturated with NaCl, dried ($MgSO_4$), and concentrated in vacuo to give 1.02 g of crude ketone **14** as a dark glass. Chromatography on silica gel using 50/50 benzene/heptane afforded 680 mg (72.5%) of *m*-methoxyphenyl 1-naphthyl ketone (**14**) as a light yellow oil (lit. (*4*) bp 260–216 °C (24 mmHg)). The infrared spectrum was identical with that of material obtained in 65% yield from the condensation of 1-cyanonaphthalene and *m*-methoxyphenylmagnesium bromide. To obtain a solid derivative for melting point comparisons, samples of ketone **14** from the decarboxylation and from the condensation were reduced with $NaBH_4$ in methanol to obtain samples of the corresponding alcohol **15,** mp 120–121 °C. Mixture melting point of samples from the above sources was not depressed, and IR spectra were superimposable. Anal. ($C_{18}H_{16}O_2$) C, H.

Structure Proof of 2-(1-Naphthoyl)-3-methoxybenzoic Acid (7a). Similarly, the isomeric keto acid **7a** was decarboxylated in 42% yield to give *o*-methoxyphenyl 1-naphthyl ketone (**14a**) which on crystallization from heptane afforded the analytical sample as lustrous colorless plates, mp 75–76 °C (lit. (*10*) mp 75–76 °C). Anal. ($C_{18}H_{14}O_2$) C, H. The above was identical in all respects with synthetic material obtained from the condensation of 1-cyanonaphthalene and *o*-methoxyphenylmagnesium bromide; IR spectra were superimposable, and mixture melting point of the two samples was not depressed.

3-Methyl-3-(1-naphthyl)-7-methoxyphthalide (8). To a refluxing solution of 3.8 g (12.4 mmol) of 2-(1-naphthoyl)-6-methoxybenzoic acid (**7**) in 50 ml of anhydrous benzene was added an ethereal solution of methylmagnesium iodide prepared from 5.3 g (37.4 mmol) of methyl iodide and 0.91 g (37.4 mmol) of magnesium. The resultant yellowish suspension was refluxed for 4 h and left to stir overnight at room temperature. The reaction mixture was poured into a mixture of ice and 10% HCl, extracted with ethyl acetate, and washed with water. The organic solution was then extracted with 5% K_2CO_3, washed with water followed by saturated salt solution, dried ($MgSO_4$), and concentrated in vacuo to give 3.07 g (81.5%) of crude product as a dark yellow foam. Crystallization from ethanol afforded analytically pure 3-methyl-3-(1-naphthyl)-7-methoxyphthalide (**8**), mp 183–184 °C. Anal. ($C_{20}H_{16}O_3$) C, H.

3-Methyl-3-(1-naphthyl)-4-methoxyphthalide (8a). Under identical conditions with those described above, the isomeric acid **7a** was converted to the phthalide **8a** in 75.5% yield. The analytically pure material was obtained by crystallization from ethanol to give a yellow solid, mp 159–161 °C. Anal. ($C_{20}H_{16}O_3$) C, H.

2-[α-1′-(Naphthyl)ethyl]-6-methoxybenzoic Acid (9). A mixture of 1.2 g (3.94 mmol) of 3-methyl-3-(1-naphthyl)-7-methoxyphthalide (**8**) and 2 g of NaOH in 20 ml of ethanol and 2 ml of H_2O was refluxed for 1 h. The reaction mixture was then partially concentrated in vacuo to remove some of the ethanol and added to 5 g of Zn(Cu) couple (prepared as per Fieser, "Organic Reagents", Vol. I, p 1293), followed by 10 ml of 50% NaOH and 20 ml of H_2O. The mixture was refluxed for 20 h, cooled to room temperature, decanted from the metal, acidified with concentrated HCl, extracted with ethyl acetate, and washed with water. The organic solution was extracted with 5% Na_2CO_3, the latter acidified with concentrated HCl and again extracted into ethyl acetate, washed with water, dried ($MgSO_4$), and concentrated in vacuo and gave crude 2-[α-1′-(naphthyl)ethyl]-6-methoxybenzoic acid which on crystallization from ethyl acetate/heptane afforded 986 mg of analytically pure product, mp 202–203 °C. Anal. ($C_{20}H_{18}O_3$) C, H.

2-[α-1′-(Naphthyl)ethyl]-3-methoxybenzoic Acid (9a). Under identical conditions, the phthalide **8a** was reductively cleaved to the acid **9a** in 84% yield. Analytically pure 2-[α-1′-(naphthyl)ethyl]-3-methoxybenzoic acid (**9a**) was obtained by crystallization from benzene as a tan solid, mp 209–210 °C. Anal. ($C_{20}H_{18}O_3$) C, H.

8-Methoxy-12-methyl-7(12H)-benz[a]anthracenone (10). A solution of 900 mg (2.94 mmol) of the tricyclic acid **9** in 50 ml

of anhydrous HF was allowed to evaporate at room temperature for 1 h. The dark solution was then poured onto ice, extracted with ethyl acetate, washed with water 5% K_2CO_3, again with water, dried ($MgSO_4$), and concentrated in vacuo and gave 840 mg of crude tetracyclic ketone as a yellow foam. Crystallization from benzene/heptane afforded 652 mg of analytically pure 8-methoxy-12-methyl-7(12*H*)-benz[*a*]anthracenone (**10**), mp 176–178 °C. Anal. ($C_{20}H_{16}O_2$) C, H.

11-Methoxy-12-methyl-7(12H)-benz[a]anthracenone (10a). Under identical conditions to the above, the acid **9a** was cyclized to the tetracyclic ketone **10a** in 97% yield. Crystallization from benzene/heptane afforded the analytically pure material as a yellow solid, mp 154–156 °C. Anal. ($C_{20}H_{16}O_2$) C, H.

8-Methoxy-7,12-dimethylbenz[a]anthracene (11). To a refluxing solution of 600 mg (2.08 mmol) of ketone **10** in 100 ml of anhydrous benzene was added an ethereal Grignard solution of MeMgI derived from 200 mg of magnesium and 1.12 g of methyl iodide (4.0 mmol). The resultant yellowish suspension was refluxed for 1 h, cooled to room temperature, poured onto a mixture of 10% HCl and ice, extracted into ether, washed with water, dried ($MgSO_4$), and concentrated in vacuo to give the crude hydroxy derivative. This was refluxed in 100 ml of benzene in the presence of 500 mg of *p*-toluenesulfonic acid hydrate for 1 h, extracted with saturated $NaHCO_3$, washed with water, dried ($MgSO_4$), and concentrated in vacuo to give the crude benzanthracene derivative. Crystallization from benzene/heptane afforded 207 mg of analytically pure 8-methoxy-7,12-dimethylbenz[*a*]anthracene as greenish, fluorescent plates, mp 142–143 °C. Anal. ($C_2H_{18}O$) C, H.

11-Methoxy-7,12-dimethylbenz[a]anthracene (11a). Under similar conditions to the above, ketone **10a** was converted to the aromatic derivative **11a** in 83% yield. Analytically pure 11-methoxy-7,12-dimethylbenz[*a*]anthracene (**11a**) was obtained by crystallization from benzene as a yellow solid, mp 123–124 °C. Anal. ($C_{21}H_{18}O$) C, H.

8-Hydroxy-7,12-dimethylbenz[a]anthracene (12) and 8-Acetoxy-7,12-dimethylbenz[a]anthracene (13). To a stirred refluxing solution of 147 mg (0.513 mmol) of the methoxy derivative **11** in 40 ml of anhydrous benzene was added 3 ml of BBr_3 in benzene and the solution was refluxed for 1 h, then cooled, poured onto crushed ice, and extracted into ethyl acetate. The organic phase was washed with water and saturated NaCl, dried ($MgSO_4$), and concentrated in vacuo to give 172 mg of a dark glass. Repeated attempts at crystallization, charcoal treatment, and chromatography did not permit isolation of crystalline **12,** although the chromatographically homogeneous material could be used for comparison purposes in the GC as the heptafluorobutyrate ester. Acetylation of the crude phenol with equal parts of pyridine/acetic acid followed by silica gel chromatography with benzene gave the crude acetate **13.** Crystallization from ether/heptane afforded analytically pure 8-acetoxy-7,12-dimethylbenz[*a*]anthracene as yellow crystals, mp 161–162 °C. Anal. ($C_{22}H_{18}O_2$) C, H.

11-Hydroxy-7,12-dimethylbenz[a]anthracene (12a) and 11-Acetoxy-7,12-dimethylbenz[a]anthracene (13a). Similarly, BBr_3 hydrolysis of the methyl ether **11a** for a 15-min period gave the phenol **12a,** which again could not be obtained in a pure, crystalline state without significant decomposition. Conversion to the acetate **13a** was again accomplished readily under identical conditions. Analytically pure 11-acetoxy-7,12-dimethylbenz[*a*]anthracene (**13a**) was obtained by crystallization from ethanol as a yellow solid, mp 118–120 °C. Anal. ($C_{22}H_{18}O_2$) C, H.

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