TABLE I

EFFECT OF DIETHYLSTILBESTROL DI-*p*-AMINOBENZOATE ON TOTAL CELL COUNT

Treatment, ppm	Total cell count $\times 10^{-4}$
0	241.0 ± 10.9
DES,[a] 0.3	263.0 ± 16.2
DES, 3.0	154.8 ± 8.6
DES ester,[b] 0.3	245.5 ± 20.0
DES ester, 3.0	169.0 ± 4.1
DES + PABA, 0.3	255.8 ± 9.6
DES + PABA, 3.0	203.8 ± 11.9
PABA,[c] 0.3	249.3 ± 23.1
PABA, 3.0	248.0 ± 4.3

[a] Diethylstilbestrol. [b] Diethylstilbestrol di-*p*-aminobenzoate. [c] *p*-Aminobenzoic acid.

The ^{14}C-leucine incorporation into cellular protein was increased when DES, DES + PABA, and DES concentration was 0.3 ppm but decreased as the concentration was increased to 3 ppm (Table II). The

TABLE II

EFFECT OF DIETHYLSTILBESTROL DI-*p*-AMINOBENZOATE ON PROTEIN SYNTHESIS

Treatment, ppm	Specific act., cpm/mg of protein
0	4299.8 ± 183.4
DES,[a] 0.3	5756.4 ± 445.1
DES, 3.0	3269.5 ± 223.0
DES ester,[b] 0.3	6216.2 ± 288.1
DES ester, 3.0	2933.2 ± 218.3
DES + PABA, 0.3	5395.4 ± 647.4
DES + PABA, 3.0	2814.7 ± 428.4
PABA,[c] 0.3	4832.8 ± 527.9
PABA, 3.0	4778.3 ± 106.3

[a] Diethylstilbestrol. [b] Diethylstilbestrol di-*p*-aminobenzoate. [c] *p*-Aminobenzoic acid.

effect of DES ester at 0.3 ppm was not different from that of DES and DES + PABA. PABA at 0.3 and 3.0 ppm showed no effect on protein synthesis. These results would indicate that the effect of DES ester is attributed to the DES moiety of the molecule.

DES stimulates the activity of several enzymes at low concentrations but the activity is decreased at higher concentrations.[5-9] Hence, the increase in protein synthesis in the present study may be the result of increases in tissue enzyme activities.

Experimental Section

All melting points were determined using a Thiele melting point apparatus. Nitrogen content was determined by Nesslerization.[10] The uv spectra were determined over the range of 300–500 mμ on a Hitachi-Perkin-Elmer spectrophotometer. The esterification of DES resulted in a bathochromic shift.

***p*-Thionylaminobenzoyl chloride (2)** was prepared by the methods described by Graf and Langer[11] and McMaster and Altmann.[12] $SOCl_2$ was refluxed by dry *p*-aminobenzoic acid at 75° for 2 hr and finally **2** was purified by vacuum distillation.

Diethylstilbestrol Di-*p*-aminobenzoate (DES Ester) (3).—The preparation of the PABA ester of DES was based on the Schotten–Baumann reaction of acylating two phenolic groups of DES with **2** in dry pyridine, then converting the thionylamino groups to amino groups in H_2O.[11,13] The dry crude product was purified by recrystallization from a mixture of DMF and H_2O (1:2, v/v), washed with Et_2O, and finally dried at 60° in a vacuum oven until a constant weight was obtained; yield 80%; mp 195°. *Anal.*[14] ($C_{33}H_{30}N_2O_4$) N.

Biological Activity.—Mouse fibroblastic cells (L2071) is a laboratory line maintained in the Carver Research Foundation Laboratory from the original strain which was received from Dr. Wilton Earl and described by McQuilkin, *et al.*[15] These cells have been maintained as a separate line by serial transfer over a period of more than 8 years before storage under liquid nitrogen.

Stock solutions of diethylstilbestrol (DES), diethylstilbestrol di-*p*-aminobenzoate (DES ester), and *p*-aminobenzoic acid (PABA) were prepared by dissolving 12.5 and 125 mg of each compound in a mixture of 0.5 ml of N,N-dimethylacetamide and 4.5 ml of Tween 80. After filtration through a Millipore filter (0.5-μ pore), 0.1 ml of each solution was added to 100 ml of culture medium (Eagles' medium 90% plus calf serum 10%) to produce solutions of 2.5 and 25 ppm concentrations, respectively. Each stock solution (24 ml) was made up to 100 ml with culture medium to produce solutions of 0.6 and 6 ppm concentrations. DES plus PABA solutions were prepared by mixing equal volumes of equivalent concentrations of DES and PABA solutions. Solutions containing DMAC and Tween 80, but without DES and DES ester, served as controls.

Each solution (5 ml) was added to an accurately determined number of cells (1.5×10^6) in 5 ml of culture medium to give final concentrations of 0, 0.3, and 3 ppm of DES, DES ester, PABA, or DES + PABA. Before incubation at 36° for 48 hr, 0.2-μCi of uniformly labeled L-leucine-^{14}C (specific activity 200 mCi/mμ) was introduced into each culture.

At the end of the incubation period the cells were centrifuged and then washed and resuspended in 10 ml of Hank's balanced salt solution. A 2.0-ml aliquot of the evenly dispersed cellular suspension was used for a radioactivity count by the method described by Bruno and Christian[16] using a Tri-carb liquid scintillation spectrophotometer. The N content in the remaining 8-ml portion was determined by Nesslerization.[10]

Each experiment was run in duplicate and repeated three times. The average value and standard deviation of the mean were calculated.

(5) R. Glass, J. Loring, J. Spencer, and C. Villee, *Endocrinology*, **68**, 327 (1961).
(6) J. C. Warren, D. O. Carr, and S. Grisolia, *Biochem. J.*, **93**, 409 (1964).
(7) W. D. Noteboom and J. Gorski, *Proc. Natl. Acad. Sci. U. S.*, **50**, 250 (1963).
(8) C. Kidson and K. S. Kirby, *Nature*, **203**, 599 (1964).
(9) T. H. Hamilton, *Proc. Natl. Acad. Sci. U. S.*, **49**, 373 (1963).
(10) F. C. Koch and T. L. McMeekin, *J. Am. Chem. Soc.*, **46**, 2066 (1924).
(11) R. Graf and W. Langer, *J. Prakt. Chem.*, **148**, 161 (1937).
(12) L. McMaster and T. F. Altmann, *J. Am. Chem. Soc.*, **50**, 145 (1928).
(13) L. Anschutz and H. Boedeker, *Ber. Deut. Chem. Ges.*, **62**, 826 (1929).
(14) Analytical result obtained is within ±0.4% of the theoretical value.
(15) W. T. McQuilkin, V. J. Evans, and W. R. Earle, *J. Natl. Cancer Inst.*, **19**, 885 (1957).
(16) G. A. Bruno and J. E. Christian, *Anal. Chem.*, **33**, 1216 (1961).

Adrenocorticolytic Derivatives of Benz[*a*]anthracene[1]

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The potent carcinogen, 7,12-dimethylbenz[*a*]anthracene (7,12-DMBA), is unique among the carcinogenic polycyclic aromatic hydrocarbons by causing destruction of two zones of the rat's adrenal, selectively.[2] Boyland and Sims[3] showed that 7,12-DMBA is metabolized in rat liver principally to the 7-hydroxymethyl (**1**) and 12-hydroxymethyl (**2**) derivatives. Later, Boyland, Sims, and Huggins[4] found that **1** is

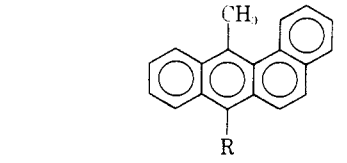
(1) This investigation was supported by grants from American Cancer Society, Jane Coffin Childs Memorial Fund for Medical Research, and Daisy Schwimmer Memorial Fund.
(2) C. Huggins and S. Morii, *J. Exptl. Med.*, **114**, 741 (1961).
(3) E. Boyland and P. Sims, *Biochem. J.*, **95**, 780 (1965).
(4) E. Boyland, P. Sims, and C. Huggins, *Nature*, **207**, 816 (1965).

several times more effective in provoking adrenal damage than the parent hydrocarbon, and Wheatley and coworkers[5] presented conclusive evidence that this metabolite and not the precursor 7,12-DMBA was the active adrenocorticolytic agent. To gain additional information concerning the structural requirements for induction of adrenal necrosis and to correlate carcinogenicity and adrenocorticolysis of derivatives of benz[*a*]anthracene, we synthesized a series of compounds related to 7,12-DMBA. The results of the biological evaluation of the new compounds have been presented elsewhere.[6] Here, we wish to relate our work concerned with their synthesis.

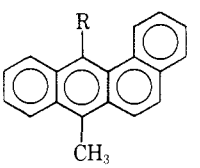
Compounds **1a** and **1b** were prepared from 7-hydroxymethyl-12-methylbenz[*a*]anthracene (**1**) by conventional methods. The 7-formyl derivative (**1c**) was first synthesized by Badger and Cook[7] from 12-methylbenz[*a*]anthracene in low yield. As relatively large amounts of this compound were needed for biological experiments and further transformation, we found that it was more convenient to prepare **1c** by oxidation of **1** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).[8] The reaction proceeded overnight at room temperature to give **1c** in better than 80% yield. In the case of **2**, oxidation of the sterically hindered 12-hydroxymethyl group was rather sluggish and after 5 days at room temperature the yield of **2a** was only 50%. At higher temperatures, a complex mixture of products resulted, as determined by tlc, which was not further investigated. The great difference in reactivity at positions 7 and 12 allowed us the selective transformation of 7,12-dihydroxymethylbenz[*a*]anthracene (**3**)[9] into **3a**. The aldehyde proton signal of **3a** appeared at the same low field as that of **1c**. The conversion of **3a** into the dialdehyde **3b** proved to be more difficult than expected. After 4 days, only traces of the dialdehyde were formed. Even after a reaction time of 12 days, **3b** could be isolated in a yield of only 10%, while about 70% of the 7-monoaldehyde was recovered unchanged.

The alcohols **1d** and **1e** were prepared from the aldehyde **1c** by Grignard reaction with MeMgBr and EtMgBr, respectively. Compound **1e** could not be obtained in crystalline form and was purified as the acetate **1f**. 7-Isobutyl-12-methylbenz[*a*]anthracene (**1g**) was synthesized from 12-methylbenz[*a*]anthr-7-one[10] and *i*-BuMgBr. The same ketone was used to prepare the 7-acetoxy derivative **1h**, which in turn served as starting material for the ethers **1i** and **1j**, using the method of Fieser and Hershberg.[11] Reaction of **2a** with MeMgBr gave 7-methyl-12-(1-hydroxyethyl)benz[*a*]anthracene (**2b**).

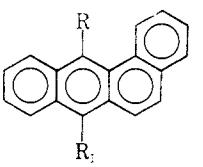
The synthesis of 7-methyl-12-ethylbenz[*a*]anthracene (**5**) was first reported by Mikhailov and Blokhina.[12] They claim to have isolated this compound directly from the reaction of 12-ethylbenz[*a*]anthr-7-one with MeMgBr. In repeating this reaction, a strong OH band appeared in the ir spectrum of the crude reaction product. However, on dehydration with $POCl_3$ and pyridine, a crystalline compound was isolated which differed from the hydrocarbon of the Russian authors. The nmr spectrum of the new compound revealed the presence of two vinylic protons and that of a tertiary aliphatic proton adjacent to two aromatic rings. Consequently, no aromatization occurred during dehydration and the compound formed was 7-methylene-12-ethyl-7,12-dihydrobenz[*a*]anthracene (**4**). The



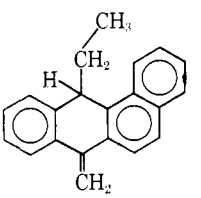
1, R = CH_2OH
1a, R = CH_2Cl
1b, R = CH_2Br
1c, R = CHO
1d, R = $CHOHCH_3$
1e, R = $CHOHCH_2CH_3$
1f, R = $CH(OCOCH_3)CH_2CH_3$
1g, R = $CH_2CH(CH_3)_2$
1h, R = $OCOCH_3$
1i, R = OCH_3
1j, R = OC_2H_5



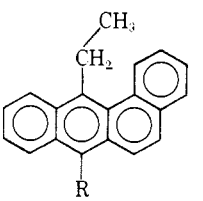
2, R = CH_2OH
2a, R = CHO
2b, R = CH_3CHOH



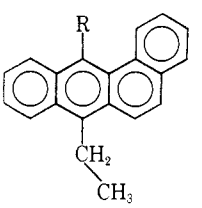
3, R = R_1 = CH_2OH
3a, R = CH_2OH; R_1 = CHO
3b, R = R_1 = CHO



4



5, R = CH_3
5a, R = CH_2OH
5b, R = CHO



6, R = CH_3
6a, R = CH_2OH

stability of **4** is due to the strong steric interaction between the ethyl group and the hydrogen at C-1 which ensues when the ring bearing the ethyl substituent becomes aromatic. Brief treatment of **4** with acid gave 7-methyl-12-ethylbenz[*a*]anthracene in quantitative yield.

Lead tetraacetate oxidation of **5** and subsequent hydrolysis of the primarily formed acetate led to the alcohol **5a**, in about 50% yield. The hydroxy compound was smoothly converted to the aldehyde **5b** with DDQ. Similar to the oxidation of **5**, $Pb(OAc)_4$ treatment of 7-ethyl-12-methylbenz[*a*]anthracene (**6**),[10] followed by hydrolysis, produced 7-ethyl-12-hydroxymethylbenz[*a*]anthracene (**6a**). The structures as-

(5) D. N. Wheatley, I. R. Kernohan, and A. R. Currie, *Nature*, **211**, 387 (1966).
(6) J. Pataki and C. B. Huggins, *Biochem. Pharmacol.*, **16**, 607 (1967).
(7) G. M. Badger and J. W. Cook, *J. Chem. Soc.*, 409 (1940).
(8) (a) E. A. Braude, R. P. Linstead, and K. R. H. Wooldridge, *ibid.*, 3070 (1956); (b) J. C. Orr, O. Halpern, P. G. Holton, F. Alvarez, I. Delfin, A. de la Roz, A. M. Ruiz, and A. Bowers, *J. Med. Chem.*, **6**, 166 (1963).
(9) G. M. Badger and J. W. Cook, *J. Chem. Soc.*, 802 (1939).
(10) B. M. Mikhailov and N. G. Chernova, *Compt. Rend. Acad. Sci. USSR*, **20**, 579 (1938); *Chem. Abstr.*, **33**, 5842[9] (1939).
(11) L. F. Fieser and E. B. Hershberg, *J. Am. Chem. Soc.*, **60**, 1893 (1938).
(12) B. M. Mikhailov and A. N. Blokhina, *J. Gen. Chem. USSR*, **10**, 1793 (1940); *Chem. Abstr.*, **35**, 4007[7] (1941).

signed to **5a** and **6a** were confirmed by their nmr spectra. Formation of products hydroxylated in the ethyl side chains could not be detected in either case.

Summarizing briefly the results of the biological evaluation,[6] only five of the compounds, **1**, **1a**, **1c**, **1d**, and **1e**, were effective in provoking adrenal necrosis in female Sprague–Dawley rats. Compared to 7,12-DMBA, the carcinogenicity of **1** was greatly reduced, while **1d** and **1e** were devoid of activity, in male rats of Long-Evans strain.[13]

Experimental Section[16]

7-Chloromethyl-12-methylbenz[*a*]anthracene (1a).—To a suspension of 1.00 g (3.68 mmoles) of 7-hydroxymethyl-12-methylbenz[*a*]anthracene (**1**)[3] in 20 ml of anhydrous C_6H_6 1.0 ml (1.65 g, 13.8 mmoles) of $SOCl_2$ was added. The crystals dissolved on stirring. The solution was refluxed for 30 min under anhydrous conditions. Excess reagent and the solvent were removed under reduced pressure. The residue (1.10 g) gave (from C_6H_6) 0.74 g of **1a**, mp 137–139° dec. The analytical sample showed mp 139–140° dec. *Anal.* ($C_{20}H_{15}Cl$) C, H, Cl.

7-Bromomethyl-12-methylbenz[*a*]anthracene (1b).—A solution of 3.97 g (14.6 mmoles) of **1** in 80 ml of anhydrous C_6H_6 was heated under reflux with 4.56 g (16.8 mmoles) of PBr_3 for 1 hr. The solution was chilled in ice–water and any excess reagent was decomposed by dropwise addition of H_2O. A crystalline material separated which was filtered off, washed (C_6H_6), and dried. The product (2.67 g) melted at 138–141° dec. From the benzene layer of the filtrate additional material (1.49 g) melting at 136–140° dec was obtained on concentration. Recrystallization of the pooled crystalline fractions from C_6H_6 yielded analytically pure bromide (2.91 g), mp 140–142° dec, and a second crop of 0.74 g, mp 139–141° dec. *Anal.* ($C_{20}H_{15}Br$) C, H, Br.

7-Methyl-12-formylbenz[*a*]anthracene (2a).—To a stirred solution of 3.05 g (11.0 mmoles) of 7-methyl-12-hydroxymethylbenz[*a*]anthacene (**2**)[3] in 45 ml of anhydrous dioxane 3.45 g (14.5 mmoles) of DDQ was added. The solution was stirred for 6 hr, then set aside for 116 hr at room temperature. The precipitated hydroquinone was removed by filtration. The filtrate was diluted with 250 ml of CH_2Cl_2 and washed several times (5% NaOH H_2O). The residue was passed through a column of 60 g of neutral alumina which was then washed (CH_2Cl_2). The crystalline residue from the evaporation of the combined solutions was recrystallized twice (Me_2CO) and gave 1.52 g (50%) of pure **2a**, mp 128–130°. The analytical sample melted at 129–131°; nmr, 175 (s, 3, CH_3), 619 cps (s, 1, 12-CHO). *Anal.* ($C_{22}H_{14}O$) C, H.

7-Formyl-12-methylbenz[*a*]anthracene (1c).[7]—Compound 1 (3.55 g, 13.0 mmoles) was oxidized with DDQ (3.55 g, 15.6 mmoles) in 60 ml of anhydrous dioxane for 16 hr at room temperature. The reaction mixture was worked up in the same manner as for **2a**; yield 2.93 g (82.7%) of **1c**; mp 112–114° after crystallization from CH_2Cl_2–Et_2O, melting point of the analytical sample 114–115° (lit.[7] mp 111.5–112.5°); nmr, 187.9 (s, 3, CH_3), 673.9 cps (s, 1, 7-CHO). *Anal.* ($C_{20}H_{14}O$) C, H.

7-(1-Hydroxyethyl)-12-methylbenz[*a*]anthracene (1d).—To a stirred solution of 1.78 g of MeMgBr in 20 ml of anhydrous Et_2O and 10 ml of anhydrous C_6H_6 cooled in an ice bath, 1.57 g of **1c** was added in one portion. In a few minutes a clear solution resulted, then stirring was continued for 4 hr at room temperature. The reaction mixture was worked up in the usual way. The evaporation residue gave, after three crystallizations from MeOH, 0.92 g of pure **1d**, mp 124–126°. *Anal.* ($C_{21}H_{18}O$) C, H.

7-(1-Acetoxypropyl)-12-methylbenz[*a*]anthracene (1f).—7-Formyl-12-methylbenz[*a*]anthracene (1.88 g) was stirred with 3.2 g of EtMgBr in 10 ml of anhydrous Et_2O and 15 ml of anhydrous C_6H_6 for 5 hr at room temperature. After the usual work-up, evaporation of the solvents left 2.02 g of a yellow foam which did not crystallize.

The crude carbinol **1e** was acetylated with 5 ml of Ac_2O and 5 ml of pyridine at room temperature overnight. The evaporation residue (2.27 g) crystallized from Et_2O–MeOH to give 1.71 g of **1f**, mp 89–95°; two recrystallizations from MeOH raised the melting point to 94–96°; on tlc plate the compound appeared as one spot; nmr, 56.8 (t, 3, CH_3CH), 122.9 (s, 3, CH_3COO), 141.8 (m, 2, $CHCH_2CH_3$), 196.6 (s, 3, CH_3), 432.2 cps (t, 1, CH_3COO-$CHCH_2$). *Anal.* ($C_{24}H_{22}O_2$) C, H.

7-(1-Hydroxypropyl)-12-methylbenz[*a*]anthracene (1e).—Compound **1f** (1.2 g) was dissolved in 12.5 ml of THF and the solution was diluted with 20 ml of 60% MeOH containing 0.5 g of KOH. The solution was stirred under N_2 for 3 hr at room temperature. Water (150 ml) was then added, and the mixture was extracted (Et_2O). The extracts were washed (H_2O) and dried, and the ether was removed under reduced pressure. The alcohol **1e** was obtained as a light yellow, foamy product which could not be crystallized. For analysis, it was dissolved in ether and precipitated by addition of MeOH as an amorphous solid, melting around 120°. *Anal.* ($C_{22}H_{20}O$) C, H.

7-Methyl-12-(1-hydroxyethyl)benz[*a*]anthracene (2b).—To a solution of 2.50 g of MeMgBr in 25 ml of anhydrous Et_2O and 20 ml of anhydrous C_6H_6, 2.00 g of **2a** was added at once. The crystals dissolved quickly. Stirring was continued for 22 hr at room temperature. After the usual work-up, the dried organic layer was concentrated and cooled. The crystalline product (2.06 g) was recrystallized from Me_2CO and gave 1.91 g of analytically pure **2b**, mp 203.5–204.5°. *Anal.* ($C_{21}H_{18}O$) C, H.

7-Formyl-12-hydroxymethylbenz[*a*]anthracene (3a).—The solution of 2.89 g (10 mmoles) of diol **3**[9] and 2.72 g (12 mmoles) of DDQ in 112 ml of anhydrous dioxane was stirred for 23 hr. The precipitated hydroquinone was filtered off and washed well (Et_2O). More ether was added to the filtrate and it was then washed (2% NaOH, H_2O, saturated NaCl). After drying (Na_2SO_4), the solution was filtered through a column of 30 g of alumina which was washed with 400 ml of ether. The combined solutions were evaporated *in vacuo*. The residue (2.81 g) gave from EtOAc 1.97 g of **3a**, mp 139–143°. Additional material (0.30 g, mp 135–140°) was obtained from the mother liquor. After two recrystallizations, the analytical sample melted at 143–144.5°; nmr (in DMSO-d_6), 162.1 (m, 1, CH_2OH), 318.5 (s, 2, 12-CH_2OH), 681.1 cps (s, 1, 7-CHO). *Anal.* ($C_{20}H_{14}O_2$) C, H.

7,12-Diformylbenz[*a*]anthracene (3b).—The hydroxyaldehyde **3a** (2.05 g, 7.2 mmoles) was dissolved in 100 ml of anhydrous dioxane. The solution was stirred with 2.05 g (9.0 mmoles) of DDQ for 20 hr and then allowed to stand for 12 days at room temperature. A 0.5% NaOH solution (600 ml) was added to the filtered solution and the mixture was extracted (EtOAc). The extract was washed (H_2O saturated NaCl) and dried (Na_2SO_4). The solvent was then evaporated. The residue (2.03 g) was dissolved in boiling absolute EtOH and the solution was concentrated to about 100 ml. On cooling, 0.21 g of a product, mp 189–194.5°, crystallized. The volume of the mother liquors was reduced to about 50 ml and 0.10 g of material melting at 178–191° was recovered. The combined crystals gave from absolute EtOH 0.22 g of pure **3b**, mp 201.5–203.5°; the melting point did not change on further crystallization; nmr (saturated in DMSO-d_6), 628.9 (s, 1, 12-CHO), 684.1 cps (s, 1, 7-CHO). *Anal.* ($C_{20}H_{12}O$) C, H.

From the mother liquors 1.38 g of starting material was recovered, after evaporation of the alcohol and crystallization of the residue from EtOAc.

7-Methylene-12-ethyl-7,12-dihydrobenz[*a*]anthracene (4).—12-Ethylbenz[*a*]anthr-7-one[12] (5.00 g) dissolved in 120 ml of anhydrous C_6H_6 was added to a solution of 12.0 g of MeMgBr in 100 ml of ether over a period of 30 min at room temperature. After stirring for 22 hr, the reaction flask was cooled in ice and 40 ml of saturated NH_4Cl solution was added dropwise. The organic layer was separated and washed (dilute HCl, H_2O)

(13) Recently, Flesher and coworkers[14] reported that the carcinogenicity of **1**, administered by gastric instillation to female Sprague–Dawley rats, approximated that of 7,12-DMBA. Boyland and Sims[15] found that both **1** and **2** are strong carcinogens when injected subcutaneously into C57 black strain mice (male and female). This again points to the necessity of exercising caution when comparing carcinogenic activities obtained by different methods in different species, or even strains.

(14) J. W. Flesher, S. Soedigdo, and D. R. Kelley, *J. Med. Chem.*, **10**, 932 (1967).

(15) E. Boyland and P. Sims, *Intern. J. Cancer*, **2**, 500 (1967).

(16) Melting points were determined in open capillary tubes on a Buchi-Tottoli melting point apparatus and are not corrected. The nmr spectra were obtained on a Varian A-60 instrument in deuteriochloroform with TMS as internal standard, when not otherwise indicated. The EtMgBr and MeMgBr reagents were analyzed commercial solutions diluted to the desired concentrations with the appropriate solvents. All Grignard reactions were run under N_2. Microanalyses were performed by Mr. J. Alicino, Metuchen, N. J. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

After drying (Na_2SO_4), the solvents were evaporated under reduced pressure. The gummy residue (5.14 g), which showed a strong OH ir band, was dissolved in 30 ml of anhydrous pyridine, the solution was chilled in ice, and 3.0 ml of $POCl_3$ was added cautiously. After standing for 20 hr at room temperature, the solution was poured on 250 g of crushed ice and the mixture was extracted (Et_2O). The extract was washed (dilute HCl, H_2O) and the ether was evaporated. The residue (4.45 g) gave from EtOH 2.09 g of **4**, mp 108–110°; the melting point did not change on recrystallization; nmr, 46.4 (t, 3, $CHCH_2CH_3$), 106.5 (m, 2, $CHCH_2CH_3$), 280.6 (t, 1, $CHCH_2CH_3$), 342.8 (s, 1, vinylic H), 345.7 cps (s, 1, vinylic H). *Anal.* ($C_{21}H_{18}$) C, H.

After treating the mother liquors of **4** with HCl, 0.78 g of 7-methyl-12-ethylbenz[*a*]anthracene (**5**),[12] mp 76–78°, could be isolated (lit.[12] mp 76–77°). Pure **4** was isomerized to its aromatic isomer **5** in quantitative yield by allowing its acetone solution to stand overnight with catalytic amounts of *p*-toluenesulfonic acid.

7-Hydroxymethyl-12-ethylbenz[*a*]anthracene (5a).—The solution of 6.40 g of **5** in 225 ml of glacial AcOH was stirred at 53° in an oil bath. Maintaining the temperature, a solution of 11.10 g (1.05 mole equiv) of $Pb(OAc)_4$ in 350 ml of AcOH was added over a period of 75 min. After stirring 30 min more, the warm solution was poured into 5 l. of ice water. The amorphous precipitate (7.78 g) was collected by filtration, dried, and chromatographed over 200 g of neutral alumina. Ether eluted 5.40 g of a partly crystalline material which was refluxed in 250 ml of a 1% methanolic KOH solution for 15 min. The warm solution was filtered from a small amount of resinous material, 2.5 ml of AcOH was added, and the solution was boiled down to about 40 ml. On cooling, 3.36 g of **5a**, mp 144–147°, crystallized. After two recrystallizations from MeOH, the compound melted at 146.5–148°; nmr, 103.0 (t, 3, CH_2CH_3), 115.9 (s, 1, OH), 225.6 (q, 2, CH_2CH_3), 325.3 cps (s, 2, CH_2OH). *Anal.* ($C_{21}H_{18}O$) C, H.

7-Formyl-12-ethylbenz[*a*]anthracene (5b).—One gram of **5a** was oxidized in 16 ml of anhydrous dioxane with 1.00 g (1.2 mole equiv) of DDQ for 17 hr at room temperature. The reaction mixture was worked up as for **2a**. The material obtained after filtration through alumina weighed 0.97 g and gave 0.84 g of **5b** when crystallized from hexane. The analytical sample melted at 92–94°; nmr, 100.9 (t, 3, CH_2CH_3), 230.1 (q, 2, CH_2CH_3), 680.2 cps (s, 1, CHO). *Anal.* ($C_{21}H_{16}O$) C, H.

7-Ethyl-12-hydroxymethylbenz[*a*]anthracene (6a).—To the stirred solution of 0.36 g of 7-ethyl-12-methylbenz[*a*]anthracene[16] in 14 ml of AcOH 0.62 g (1.05 mole equiv) of $Pb(OAc)_4$ in 20 ml of AcOH was added in the course of 80 min. A temperature of 58° was maintained throughout the addition and 30 min after. The warm solution was poured into 350 ml of ice water. The amorphous precipitate was filtered off, dried, and dissolved in ether. The solution was filtered through a column of 10 g of alumina. The residue from the evaporation of the filtrate was heated under reflux in 30 ml of a 1% methanolic KOH solution for 15 min. The solution was neutralized (AcOH) and the solvent was evaporated *in vacuo*. The oily residue (0.29 g) crystallized from a small amount of MeOH; yield 0.15 g of **6a**; mp 151–153°; nmr, 83.8 (t, 3, CH_2CH_3), 137.4 (s, 1, OH), 213.5 (q, 2, CH_2CH_3), 329.8 cps (s, 2, CH_2OH). *Anal.* ($C_{21}H_{18}O$) C, H.

7-Isobutyl-12-methylbenz[*a*]anthracene (1g).—12-Methylbenz[*a*]anthr-7-one[16] (5 g) was added to a stirred solution of *i*-BuMgBr prepared from 24 g of *i*-BuBr in 45 ml of Et_2O and 85 ml of C_6H_6. The solution was stirred at room temperature for 19 hr and then heated under reflux for 1 hr. The reaction mixture was worked up as for **4**. The ir spectrum of the residue (5.80 g) obtained from the evaporated solvents showed an OH band. To dehydrate the intermediate carbinol, the material was dissolved in 100 ml of C_6H_6 and the solution was refluxed with 1.0 g of *p*-toluenesulfonic acid for 1 hr. The cooled solution was washed (dilute Na_2CO_3, H_2O). After drying, the solvent was removed. The residue (5.71 g) was chromatographed over 150 g of alumina. Petroleum ether eluted 2.78 g of a faintly yellow oil which crystallized from EtOH and gave 1.96 g of **1g**, mp 77–80°; after two recrystallizations from EtOH, mp 80–81°. *Anal.* ($C_{23}H_{22}$) C, H.

Crystalline material (1.5 g) was eluted with a mixture of petroleum ether (bp 30–60°)–ether (9:1). Recrystallization from hexane gave 1.18 g of pure 12-methylbenz[*a*]anthracene, mp 137–139° (lit.[10] mp 138–139°).

7-Acetoxy-12-methylbenz[*a*]anthracene (1h).—A solution of 6.0 g of 12-methylbenz[*a*]anthr-7-one[10] in 30 ml of AcOH and 30 ml of Ac_2O was heated under reflux with 1.2 g of anhydrous $ZnCl_2$ for 2 hr. To the warm solution H_2O was added cautiously until crystallization set in. After cooling, the crystals were collected, washed with MeOH, and dried; yield 5.97 g of **1h**, mp 193.5–195°. The melting point remained unchanged on recrystallization from EtOH. *Anal.* ($C_{21}H_{16}O$) C, H.

7-Methoxy-12-methylbenz[*a*]anthracene (1i).—To a solution of 12.60 g of *n*-BuMgBr in 45 ml of Et_2O and 10 ml of C_6H_6 6.00 g of **1h** was added with stirring. The resulting solution was refluxed for 1 hr. A solution of 22.00 g of Me_2SO_4 in 150 ml of toluene was then added. The mixture was warmed to 93–94° in an oil bath for 4 hr. H_2O (100 ml) was added and the mixture was stirred at 90° for 1 hr more. After cooling, the organic layer was washed (H_2O) and dried (Na_2SO_4). Removal of the solvents left 5.94 g of an oil. This was dissolved in C_6H_6 and the solution was filtered through a column of 100 g of alumina. The column was washed with additional quantities of C_6H_6. The residue (5.02 g) obtained on evaporation of the solvent, crystallized from hexane to give 3.16 g of **1i**, mp 75–77°. The twice recrystallized material melted at 76–77.5°. *Anal.* ($C_{20}H_{16}O$) C, H.

7-Ethoxy-12-methylbenz[*a*]anthracene (1j) was prepared from **1h** in exactly the same fashion as the 7-methoxy derivative, substituting Et_2SO_4 for Me_2SO_4. Six grams of **1h** yielded 2.49 g of the ethyl ether **1j**, mp 117–119°. For analysis, the material was recrystallized from hexane and melted at 118.5–119.5°. *Anal.* ($C_{21}H_{18}O$) C, H.

Hypocholesterolemic Agents. Compounds Related to Ethyl α-(4-Chlorophenoxy)-α-methylpropionate

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In 1962 Thorp[2] reported that a combination of ethyl α-(4-chlorophenoxy)-α-methylpropionate (**1**)[3] and androsterone reduces serum lipid levels in experimental animals. Later, comparative studies on the mixture and **1** alone showed both to be equally effective in lowering elevated serum cholesterol levels and probably also in reducing elevated triglycerides.[4] Following these reports the hypocholesterolemic and hypolipidemic effects of **1** have been the subjects of numerous publications,[5-7] but the mechanism of action of the compound still re-

(1) (a) To whom reprint requests and inquiries concerning this work should be directed. (b) American Foundation for Pharmaceutical Education Fellow.

(2) J. M. Thorp, *Lancet*, **1**, 1323 (1962).

(3) Clofibrate, Chlorpenisate, ICI-28257, CPIB, Atromid-S, Regelan.

(4) M. F. Oliver, *J. Atherosclerosis Res.*, **3**, 427 (1963).

(5) (a) Symposium on Atromid, *ibid.*, **3**, 341 (1963); (b) M. M. Best and C. H. Duncan, *J. Lab. Clin. Med.*, **64**, 634 (1964); (c) D. S. Platt and J. M. Thorp, *Biochem. Pharmacol.*, **15**, 915 (1966).

(6) (a) J. Engstrom, *Acta Med. Scand.*, **180**, 519 (1966); (b) L. V. Baranova, *Kardiologiya*, **5**, 65 (1965); (c) V. Kallio and L. B. Soucander, *Gerontol. Clin.*, **8**, 349 (1966); (d) R. G. Gould, E. A. Swyryd, B. J. Coan, and D. R. Avoy, *J. Atherosclerosis Res.*, **6**, 555 (1966); (e) E. D. Hocking, R. Chakrabarti, J. Evans, and C. R. Fearnley, *ibid.*, **7**, 121 (1967); (f) V. S. Zadorchenko, *Sov. Med.*, **30**, 77 (1967); (g) E. S. Argain, M. D. Bogdonoff, and C. Cain, *Arch. Internal Med.*, **119**, 80 (1967); (h) R. W. Robinson and R. J. LeBeau, *Amer. J. Med. Sci.*, **253**, 106 (1967); (i) J. W. Davis, S. J. Wilson, and J. R. McField, *ibid.*, **252**, 697 (1966); (j) J. R. O'Brien and J. B. Heywood, *Thromb. Diath. Haemorrhag.*, **16**, 768 (1966); (k) A. Herrernan, N. J. Hickey, R. Mulcahy, and R. MacFarlane, *Irish J. Med. Sci.*, **490**, 423 (1966); (l) G. Frandoli, A. Gibelli, and P. Giarola, *Clin. Terap.*, **38**, 27 (1966); (m) E. Giety, *Med. Klin.* (Munich), **62**, 336 (1967).