Stirring was continued approximately 30 min and the ethereal phase washed with ice–water and dried. Determination of peroxide content using a $Na_2S_2O_3$ titration technique,[10] indicated a 70% yield (0.032 mole) of ethyl sebacyl peroxide. The ethereal solution of peroxide V was used directly in preparation of carbostyril VI.

6-Hydroxy-5,8-dioxocarbostyril (III).—Oxidation of pyridone II in DMSO-*t*-BuOH with molecular O_2 was repeated as previously described[3] except the mixture was vigorously shaken or stirred, thereby reducing total reaction time to 3 hr. Crystallization of crude product from THF gave, in addition to quinone III, the intensely red 5,6-dioxo-8-hydroxycarbostyril (IV, 10% yield). *o*-Quinone IV exhibited λ_{max} 227, 322, and 443 mμ (log ε 4.16, 4.27, and 2.61). Both quinone III and IV were soluble in aqueous $NaHSO_3$.[11] Intraconversion of isomeric quinones III and IV was easily realized. Recrystallizing *o*-quinone IV from THF yielded *p*-quinone III. Filtering a solution of *p*-quinone III in THF through a column of silica gel provided *o*-quinone IV.

6-Hydroxy-7-(8-ethoxycarbonyloctyl)-5,8-dioxocarbostyril (VI).—Hydroxyquinone III (5.4 g, 0.03 mole) was dissolved in hot (steam bath) AcOH (600 ml). Heating and stirring were continued 2 hr while slowly adding the ethereal solution of ethyl sebacyl peroxide (0.03 mole). Simultaneously Et_2O was removed by distillation. Before completely removing solvent *in vacuo*, heating was continued another 2 hr. The residue was triturated with Et_2O and the less soluble starting material (quinone III) was collected. The ethereal filtrate was extracted with dilute $NaHCO_3$ solution and the aqueous extract was washed well with Et_2O. The basic solution was acidified with 2 *N* HCl and quinone VI was extracted with Et_2O. Following removal of solvent, the red oily residue crystallized from EtOH as orange clusters (1.5 g pure by tlc with 1:2 $CHCl_3$–MeOH as solvent). An analytical specimen was recrystallized from EtOH in the same color and crystal form: mp 162–163°; λ_{max} 234, 329, and 446 mμ (log ε 4.31, 4.35, and 2.55); ν_{max} 3180, 3120, 1778, 1740, 1680, 1645, and 1618 cm^{-1}; pmr, δ 1.27 (triplet, 3 CH_3 protons, J = 7 cps), 1.38 (broad singlet, 12 CH_2 protons), 2.30 (triplet, 2 CH_2 protons, J = 7 cps), 2.44 (triplet, 2 CH_2 protons, J = 7 cps), 4.15 (quartet, 2 $-CH_2O-$ protons, J = 7 cps), 6.84 (doublet, 1 vinyl proton, J = 9 cps), 8.01 (doublet, 1 vinyl proton, J = 9 cps), and 9.08 (complex, a 2 proton signal removed upon contact with D_2O).

Anal. Calcd for $C_{20}H_{25}NO_6$: C, 63.98; H, 6.71; N, 3.73. Found: C, 64.15; H, 6.84; N, 3.73.

5,6,8-Triacetoxy-7-(8-ethoxycarbonyloctyl)carbostyril (VII).—To a suspension of ethyl ester VI (2.0 g) in Ac_2O (4 ml) was added Zn dust (2 g) and anhydrous NaOAc (0.4 g). Stirring (magnetically) was maintained at room temperature 4 hr. AcOH (15 ml) was added and the solution was filtered. The pale yellow filtrate was diluted with H_2O (150 ml) and triacetoxyquinoline VII separated. Triacetate VII was collected, washed (H_2O), and dried; yield 2.4 g (96%). Recrystallization from 95% EtOH gave an analytical sample as a white powder melting at 164–165°; tlc, $CHCl_3$–MeOH (1:2); λ_{max} 237, 259, and 293 mμ; ν_{max} 3270, 1746, 1690, and 1660 cm^{-1}; pmr, δ 1.22 (triplet, 3 CH_3 protons, J = 7 cps), 1.32 (broad singlet, 12 CH_2 protons), 2.32 (singlet, acetate), 2.37 (singlet, acetate), 2.30 (multiplet, 4 CH_2 protons), 2.56 (singlet, acetate), 4.12 (quartet, 2 $-CH_2O-$ protons, J = 7 cps), 6.63 (doublet, 1 vinyl proton, J = 9 cps), 7.69 (doublet, 1 proton, J = 9 cps), and 11.95 (diffuse singlet, 1 proton disappearing upon equilibration with D_2O).

Anal. Calcd for $C_{26}H_{33}NO_9$: C, 62.01; H, 6.61; N, 2.78. Found: C, 62.22; H, 6.70; N, 2.90.

6-Hydroxy-5,8-dioxo-7-(9-hydroxy-9-pentyltetradecyl)carbostyril (VIII).—Triacetoxyquinoline VII (2.0 g) in THF (50 ml, alumina dried) was added during 2 hr to the Grignard reagent prepared from 1-bromopentane (8.8 g), Mg turnings (1.3 g), and dry (distilled from Na) Et_2O (50 ml). Before adding 2 *N* HCl (100 ml), stirring at room temperature was continued 15 hr. The aqueous layer was discarded and air was bubbled through the ethereal solution for 15 min. Following removal of solvent *in vacuo*, the residual oil in C_6H_6 (200 ml) was washed (five times) with 0.8% $NaHCO_3$ in 7:13 H_2O–MeOH. The combined $NaHCO_3$ extract was washed with C_6H_6 and acidified with 2 *N* HCl. Extraction with C_6H_6 and removal of solvent from the combined extract led to a red oil. Crystallization from EtOH provided an orange powder (1.3 g) homogeneous on tlc (1:2, $CHCl_3$–MeOH as solvent). One recrystallization from EtOH afforded a pure specimen as an orange powder melting at 150–151°; λ_{max} 233, 330, and 445 mμ (log ε 4.27, 4.30, and 2.52); ν_{max} 3620–3320 (broad), 3250, 1688, 1665, 1630, and 1618 cm^{-1}; pmr, δ 0.90 (triplet, 6 CH_3 protons, J = 5 cps), 1.34 (broad singlet, 30 CH_2 protons), 2.56 (poorly resolved triplet, 2 CH_2 protons), 6.88 (doublet, 1 vinyl proton, J = 9 cps), and 8.00 (doublet, 1 vinyl proton, J = 9 cps).

Anal. Calcd for $C_{28}H_{43}NO_5$: C, 71.00; H, 9.15; N, 2.96. Found: C, 70.94; H, 9.19; N, 2.88.

A Diethylstilbestrol Ester

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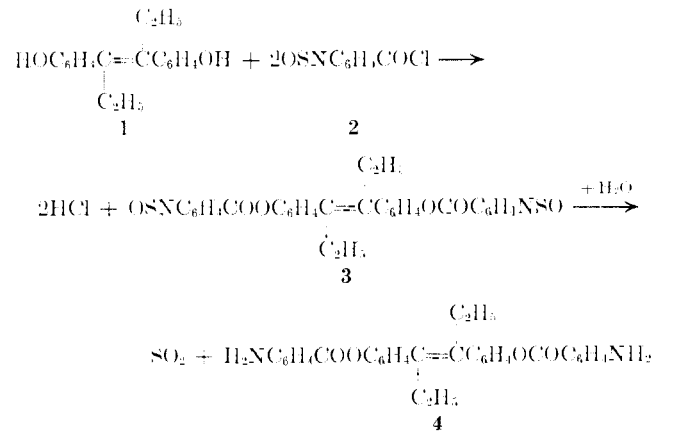
Diethylstilbestrol (DES) inhibits mitosis of chick heart fibroblastic cells[1] and epithelial growth in human prostatic adenoma and adenocarcinoma in tissue culture.[2,3] *p*-Aminobenzoic acid (PABA) has been found to inhibit or retard the enzymatic oxidation of DES and hence it was speculated that PABA has a protective or sparing action on DES.[4]

In this study the synthesis of the PABA ester of DES is reported and its effect on protein synthesis in mouse fibroblastic cells (L2071) in tissue culture is assessed.

The synthesis of di-*p*-aminobenzoyl ester of diethylstilbestrol (**4**) from 3,4-bis(*p*-hydroxyphenyl)-3-hexene (**1**) and *p*-thionylaminobenzoyl chloride (**2**) is proposed to occur after the formation of 3,4-bis(*p*-thionylaminobenzoyl-*p*-hydroxyphenyl)-3-hexene (**3**) (Scheme I).

SCHEME I



DES, DES ester, PABA, and DES + PABA at 0.3-ppm level did not affect total cell count but at 3.0-ppm level DES, DES ester, and DES + PABA decreased total cell count as compared with the 0-ppm level (Table I). PABA at 3.0 ppm exhibited no effect.

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TABLE I

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

Treatment, ppm	Total cell count × 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.

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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

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