TABLE I
19-NORTESTOSTERONE 17β-ESTERS

					Duration of myotrophic–androgenic response[a]									
					2 weeks		3 weeks		4 weeks		6 weeks		8 weeks	
No.	Ester	Mp, °C	Formula	Analyses	SV	LA	SV	LA	SV	LA	SV	LA	SV	LA
I	Cyclohexanecarboxylate	30–31	$C_{25}H_{36}O_3$		131.9	62.1			49.2	83.1	50.0	11.3		
II	Cycloheptanecarboxylate	101–106	$C_{26}H_{38}O_3$	H; C[b]	86.1	39.9	76.8	50.4	69.6	75.3	18.9	36.5	19.3	107.7
III	Cyclooctanecarboxylate	108–111	$C_{27}H_{40}O_3$	C, H	83.3	30.5	105.8	76.7	51.8	75.1	15.2	72.5	55.5	130.0
IV	α-Chlorocycloundecanecarboxylate	141–146	$C_{28}H_{43}ClO_3$	C, H, Cl	3.1	15.1	3.1	16.8	2.1	11.5				
V	Cycloundecanecarboxylate	88–89	$C_{28}H_{44}O_3$	C, H	7.8	32.8			1.8	38.5	3.9	30.7		
VI	Cyclooctaneacetate	66–68	$C_{28}H_{42}O_3$	C, H	27.1	54.1			17.8	36.8	24.8	35.0	1.8	29.7
VII	α-Chlorocyclooctaneacetate	159–160	$C_{28}H_{41}ClO_3$	C, H, Cl	(−0.9)	15.5			(−1.4)	(−1.3)	(−6.7)	10.0	11.0	5.6
VIII	2-*exo*-Norbornene-5-carboxylate	Oil	$C_{25}H_{34}O_3$	H; C[c]	6.1	3.6			7.8	35.5	1.9	26.2		
IX	Adamantaneacetate	148–153	$C_{30}H_{42}O_3$	C, H	22.6	15.6			8.1	31.8	(−0.2)	19.3		
X	Homoadamantoate	194–196	$C_{30}H_{42}O_3$	C, H	0.7	27.2			6.7	14.1	19.7	45.5	0.8	21.1

[a] The dose employed was a single subcutaneous injection of 8 mg except for esters V, IX, and X in which it was 7.5 mg. SV = seminal vesicle, LA = levator ani. Values are given as milligrams increase over control. [b] C: calcd, 78.35; found, 78.81. [c] C: calcd, 79.15; found, 78.48.

studied. While the degree of separation of myotrophic and androgenic activities in IX and X is very good, and the androgenic response is quite low at the fourth and sixth week, the anabolic potency is less than that reported for the adamantoate ester.

Experimental Section[a]

Acids.—The cycloheptane- and cycloundecanecarboxylic, and cyclooctaneacetic acids are available from Aldrich Chemical Co. Homoadamantoic and adamantaneacetic acids were prepared from adamantoic acid by the procedure of Stetter and Rauscher.[4] The *exo*-2-norbornene-5-carboxylic acid was obtained by the iodolactonization purification procedure.[5] Cyclooctanecarboxylic acid was prepared by carbonation of the cyclooctyl bromide, albeit in low yield.[6a]

Acid Chloride.—The above carboxylic acids were converted to their respective acid chlorides by means of purified $SOCl_2$.[11] A more convenient procedure which gave the cyclooctanecarbonyl chloride directly utilizing peroxide-catalyzed carboxylation with oxalyl chloride[12] in the presence of cyclooctane is described below.

Cyclooctanecarbonyl Chloride.—A solution of 100 g of cyclooctane (1.14 moles), 94 ml of redistilled oxalyl chloride (0.52 mole), and 6.6 g of recrystallized benzoyl peroxide (0.027 mole) was heated under reflux for 24 hr. Fractionation of the solution yielded 23.75 g of cyclooctanecarbonyl chloride, bp 105–115° (9 mm), yield 12.5%. The remainder of material recovered by distillation consisted of oxalyl chloride (66 ml) and cyclooctane (77 g). The yield based on recovered hydrocarbon was 67%.

α-Chlorocyclooctaneacetyl Chloride.—To 500 mg of cyclooctaneacetic acid was added 7.5 ml of aged undistilled $SOCl_2$. The solution was refluxed on the steam bath for 7 hr and then allowed to remain at room temperature overnight. Excess $SOCl_2$ was evaporated under vacuum, leaving a lightly colored residue. *Anal.* Calcd for $C_{10}H_{16}Cl_2O$: Cl, 31.77. Found: Cl, 30.65.

Steroid 17β-Cyclic Esters.—The preparation of the 17β-esters essentially followed the previously published procedure.[2] The physical constants for these compounds appear in Table I. The crystalline esters were isolated directly from the reaction mixture and in some cases were purified by chromatography.

19-Nortestosterone 17β-(α-Chlorocyclooctaneacetate).—Chromatography over Florisil of the reaction residue resulting from reaction of 7.6 g of nortestosterone and 5 g of α-chlorocyclooctaneacetyl chloride in benzene and pyridine furnished 5.2 g of ester. Recrystallization from ether gave two forms of crystalline products, mp 159–160° and 123–125°. The lower melting form had the proper analysis and the identical optical rotation and X-ray pattern as the higher melting form. Nmr data reveal 54 (18-H, s), 283 (17-H, s), 351 (4-H, s), and 248.5 (–CHCO–, d, J = 7 cps) cps.

(9) All melting points are uncorrected. The microanalyses were performed by Messrs. W. L. Brown, H. L. Hunter, and D. L. Cline. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

(10) A. S. Hussey, *J. Am. Chem. Soc.*, **73**, 1364 (1951).

(11) H. Stetter and E. Rauscher, *Chem. Ber.*, **93**, 1161 (1960).

(12) M. S. Kharasch and H. C. Brown, *J. Am. Chem. Soc.*, **64**, 329 (1942).

Synthesis of 6-Hydroxy-5,8-dioxo-7-(9-hydroxy-9′-*n*-pentyltetradecyl)carbostyril[1]

GEORGE R. PETTIT AND LEONARD E. HOUGHTON

Department of Chemistry, Arizona State University, Tempe, Arizona 85281

Received February 29, 1968

Proceeding from the weak activity shown by hydrolapachol (Ia) against *Plasmodium lophurae* in ducks, Fieser and colleagues (during the period 1942–1945) systematically and very effectively prepared a number of related napthoquinones with improved antimalarial properties.[2a] Of these, the carefully designed lapinone (Ib) offered most promise of not being easily transformed to an inactive metabolic product and inhibiting parasite respiration. Shortly after World War II, a brief clinical trial of lapinone against *Plasmodium vivax* gave encouraging results.[2b] The present and increasing need for an effective chemotherapeutic treatment of *Plasmodium falciparum* infestations in man led us to explore further the interesting lead provided by lapinone. As the important metabolic products of certain quinoline-type antimalarials appear to be quinoline-quinones and carbostyrils,[3] synthesis and antimalarial evaluation of carbostyril VIII seemed an important objective.

The ready oxidation of cyclohexyl ketone II with molecular oxygen in dimethyl sulfoxide containing potassium *t*-butoxide to hydroxyquinone III as reported[4] earlier was selected as the starting point for the synthesis of quinone VIII. Pyridone II was easily obtained by a two-step reaction sequence from 1,3-dioxacyclohexane. Accordingly, the preparation of

(1) This study received support from the U. S. Army Medical R and D Command under Contract DA-49-193-MD-3010 and the present manuscript is Contribution No. 349 from the Army Research Program on Malaria.

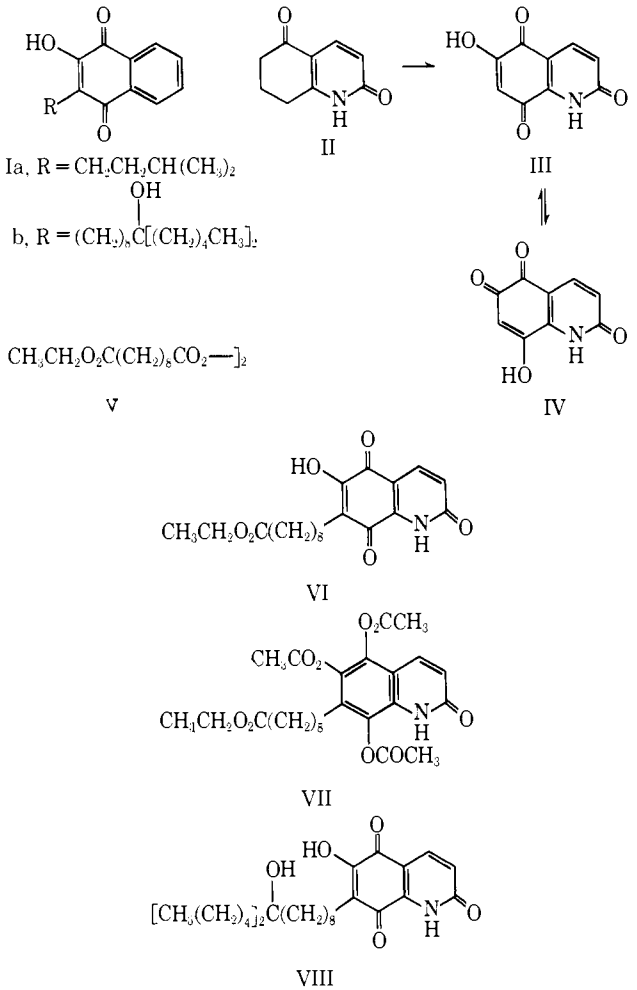
(2) (a) A most interesting review of this subject has been prepared by L. F. Fieser, "The Scientific Method," Reinhold Publishing Corp., New York, N. Y., 1964, p 163; see also, L. F. Fieser, J. P. Schirmer, S. Archer, R. R. Lorenz, and P. I. Pfaffenbach, *J. Med. Chem.*, **10**, 513 (1967); (b) G. Fawaz and L. F. Fieser, *J. Am. Chem. Soc.*, **72**, 996 (1950).

(3) Therapeutic activity of the important 6-methoxy-8-aminoquinoline antimalarials has been attributed to their *in vivo* conversion to quinoline-quinones and rabbit liver was found to convert quinine into the corresponding carbostyril derivative. References pertinent to this subject and a summary of experiments concerned with preparation of 5,6-dioxocarbostyrils will be obtained by referring to: R. R. Holmes, J. Conrady, J. Gaulrie, and R. McKay, *ibid.*, **76**, 2460 (1954).

(4) G. R. Pettit, W. C. Fleming, and K. F. Pfadl, *J. Org. Chem.*, **33**, 1189 (1968).

quinone III was repeated and in the present instance, the intensely red isomeric *o*-quinone IV was also isolated (approximately 10% yield). The following evidence seemed to favor structure IV. The intramolecularly hydrogen-bonded hydroxyl group of *p*-quinone III displays broad ir absorption between 3620 and 3400 cm^{-1}, whereas hydroxyl group absorption arising from *o*-quinone IV gives rise to a sharp absorption band at 3330 cm^{-1}. Further, the uv absorption has shifted from λ_{max} 244, 308, and 427 mμ for quinone III to λ_{max} 227, 322, and 443 mμ for quinone IV. Even more useful was the rapid intraconversion of *o*-quinone IV to *p*-quinone III merely upon recrystallization from tetrahydrofuran while orange isomer III was efficiently transformed to IV by passing in solution through a silica gel column. A future study of this isomerization at varying pH values would seem of interest.

Next, the half ethyl ester of sebacic acid was converted to acyl peroxide V. Attack by the free radical derived from an acyl peroxide has been quite useful with hydroxynaphthoquinones[5] and quinolinequinones[6] for alkylating the quinone ring. Assuming a similar alkylation course, carbostyril III was condensed in hot



glacial acetic acid with ethyl sebacyl peroxide. Particularly, pmr examination of the orange crystalline product (20% yield) clearly supported 7-alkylated carbostyril VI. The vinyl protons at positions 3 and 4 appeared as doublets at δ 6.84 and 8.01, respectively, with coupling constants of 9 cps. Other aspects of the pmr spectrum were also as anticipated. Reduction and acetylation of quinone VI with zinc dust–acetic anhydride provided (96% yield) colorless triacetate VII. Treating ethyl ester VII with *n*-pentylmagnesium bromide in THF and subjecting the crude product to oxidation with air afforded the required quinone (VIII) as an orange powder melting at 150–151°. The preceding nine-step synthesis of carbostyril VIII offers the prospect of being generally useful for obtaining such quinones.

Quinone VIII is being evaluated under direction of the Walter Reed Army Medical Center, Washington, D. C. At present, available antimalarial screening results are as follows. Employing dose regimens up to 640 mg/kg in prior (3 days) infected (*Plasmodium berghei*) mice, quinone VIII was considered inactive. A mean survival time for the treated group at least twice that of controls (7.0 ± 0.5 days) suggests potentially useful activity.

Experimental Section[7]

Ethyl Hydrogen Sebacate.—The procedure employed by Swann and colleagues[8] was modified as follows. A mixture of sebacic acid (101 g), diethyl sebacate (75 g), *n*-Bu_2O (25 ml), and concentrated HCl (12.5 ml, sp gr 1.19) was heated at reflux (reaction mixture temperature maintained at 160°) until homogeneous. The heating bath temperature was lowered to 110–120° and 95% EtOH (30 ml) was added. Heating at reflux was then continued 2 hr. An additional 10 ml of EtOH was added and heating at reflux continued 2 hr. The reaction flask was equipped for distillation and components vaporizing at water aspirator pressure and a bath temperature of 120° were removed. Cooling a solution of the residual oil in C_6H_6 caused unreacted sebacic acid (31 g) to crystallize. The C_6H_6 filtrate was concentrated *in vacuo* and the residue distilled through a 350-mm heated column packed with glass helices. Diethyl sebacate (61 g) was collected at 134–137° (1.5 mm). A pure fraction corresponding to ethyl hydrogen sebacate (33 g) boiled at 163–167° (1.5 mm) and melted at 34–35°. Crystallization of fractions boiling at 155–163 and 167–175° from hexane provided an additional 18 g of ethyl hydrogen sebacate: total yield 49%.

For preparing lesser quantities of ethyl hydrogen sebacate, the crude reaction mixture was more conveniently separated by column chromatography on silica gel. Elution with C_6H_6 gave diethyl sebacate while continued elution with 1:1 C_6H_6–$CHCl_3$ yielded ethyl hydrogen sebacate. Further purification of the half-ester by recrystallization from hexane gave an over-all yield of approximately 60%.

Ethyl Sebacyl Peroxide (V).[9]—The ethyl sebacyl chloride prepared from ethyl hydrogen sebacate (20 g) and $SOCl_2$-C_5H_5N was added to dry Et_2O (125 ml) and cooled to −5°. Cooling at −5° was continued while adding 30% H_2O_2 (6.6 ml) followed by 20% NaOH (20 ml) during 1.5 hr. The reaction mixture was treated with AcOH until weakly basic to litmus.

(5) See, for example, J. W. Taylor and J. C. Martin, *J. Am. Chem. Soc.*, **89**, 6904 (1967), and especially studies by L. F. Fieser, M. Z. Nazer, S. Archer, D. A. Berberian, and R. G. Slighter, *J. Med. Chem.*, **10**, 517 (1967), and L. F. Fieser, M. T. Leffler, *et al.*, *J. Am. Chem. Soc.*, **70**, 3206 (1948).

(6) Y. T. Pratt and N. L. Drake, *ibid.*, **82**, 1155 (1960); **77**, 4664 (1955).

(7) Solvents were redistilled and solvent extracts of aqueous solutions were dried over $MgSO_4$. Each reaction was monitored using tlc plates prepared from silica gel G (E. Merck, A. G. Darmstadt). The tlc plates were developed employing I_2. Silica gel (0.05–0.20 mm) obtained from E. Merck was used for column chromatography. Analytical specimens displayed one spot on a thin layer chromatogram. Melting points were determined using a Kofler melting point apparatus. The uv (EtOH solution, Cary-14 spectrophotometer), ir (in KBr, Beckman IR-12), and pmr ($CDCl_3$, Varian Associates, A-60 spectrometer) spectra were provided by Miss K. Reimer. Chemical shifts (δ) are relative to TMS as internal standard. Element microanalyses were performed in the laboratory of Dr. A. Bernhardt, Max Planck Institut, Mülheim, Germany.

(8) S. Swann, Jr., R. Oehler, and R. J. Buswell in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., John Wiley and Sons, Inc., New York, N. Y., 1944, p 276.

(9) This procedure is based upon one kindly provided by Dr. Richard Strube, Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D. C.

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[1] 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 $C_{26}H_{35}NO_6$: C, 65.98; H, 6.71; N, 3.73. Found: C, 64.15; H, 6.84; N, 3.75.

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} 257, 259, and 295 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

RONALD A. CHUNG, K. M. SHIH, AND RUSSEL W. BROWN

Department of Food Science and Carver Research Foundation, Tuskegee Institute, Tuskegee Institute, Alabama 36088

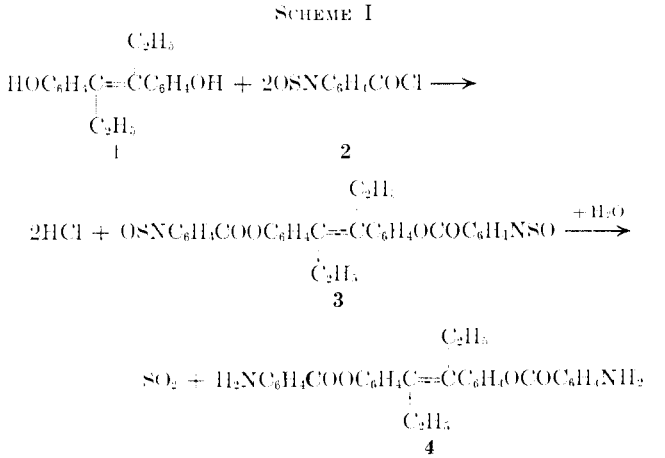
Received December 18, 1967

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.

(1) H. Lettré and H. Fernholz, *Z. Physiol. Chem.*, **278**, 201 (1943).
(2) A. Wojewski and D. P. Kaniewicz, *J. Urol.*, **93**, 721 (1965).
(3) G. S. Tarnowski, F. A. Schmid, J. G. Capraccino, and C. C. Stock, *Cancer Res.*, **26**, 181 (1966).
(4) S. Ansbacher, W. A. Wisansky, and G. J. Martin, *Federation Proc.*, **1**, 98 (1942).

(10) V. R. Kokatnur and M. Jelling, *J. Am. Chem. Soc.*, **63**, 1432 (1941).
(11) A. R. Burnett and R. H. Thomson, *J. Chem. Soc., C*, 1261 (1967).