genation of the tribromoaldehyde, 1,4-addition of the amine to the resulting dibromoacrolein followed by anil-type condensation with another molecule of the amine. The conjugate addition of the amine followed by elimination of a group from the β-carbon is quite similar to the well known ethoxymethylenemalonic ester synthesis. The final products then might arise by the ring closure of such intermediates as I or II followed by dehydrobromination to the aromatic quinoline.

Experimental[3]

Preparation of 3-Bromoquinolines.—Except as noted the amine (0.04 mole) was dissolved in 100 ml. of glacial acetic acid at steam-bath temperatures and the tribromoaldehyde was added with stirring. The reaction was completed by continued heating for three hours. After cooling and filtering, the crude hydrobromide was stirred with ammonia and the product removed by filtration, dried and crystallized from alcohol.

The nitroquinolines were reduced to the corresponding amines by stannous chloride and hydrochloric acid in the usual way.

3-BROMOQUINOLINES

5	6	8	M.p.,[a] °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
	NO_2	Cl	203–204	64	$C_9H_4BrClN_2O_2$	9.75	9.95
	NO_2	OCH_3	219–220	78	$C_{10}H_7BrN_2O_3$	9.90	9.74
	NO_2	CH_3[b]	190–191	80	$C_{10}H_7BrN_2O_2$	10.49	10.37
	NO_2	Br[c]	218–219	41	$C_9H_4Br_2N_2O_2$	8.44	8.56
	C_6H_5	NO_2[d]	183–186	79	$C_{15}H_9BrN_2O_2$	8.72	8.83
	CH_3	NO_2	190	60	$C_{10}H_7BrN_2O_2$	10.49	10.67
	CH_3	CH_3	59–60	32	$C_{11}H_{10}BrN$	5.95	6.14
	Cl	COOH	237–238	72	$C_{10}H_5BrClNO_2$	4.90	5.09
	NO_2	NO_2	157[e]	40			
NO_2		OCH_3	212–213	50	$C_{10}H_7BrN_2O_3$	9.90	9.80

AMINES

5	6	8	M.p.,[a] °C.	Yield, %	Formula	Nitrogen, % Calcd.	Found
NH_2		OCH_3	171	71	$C_{10}H_9BrN_2O$	11.43	10.87
	C_6H_5	NH_2	121–122	80	$C_{15}H_{11}BrN_2$	9.36	9.78
	CH_3	NH_2	117–118	70	$C_{10}H_9BrN_2$	11.82	11.48
	NH_2	Cl	223	77	$C_9H_6BrClN_2$	10.89	11.02
	NH_2	OCH_3	224–225	77	$C_{10}H_9BrN_2O$	11.43	11.43
	NH_2	CH_3	141–142	72	$C_{10}H_9BrN_2$	11.82	12.23

[a] All m.p.'s were taken on a Fisher–Johns block. [b] W. O. Kermack and T. W. Wright, *J. Chem. Soc.*, 1421 (1935), reported m.p. 188–189° for the compound obtained by bromination of 6-nitro-8-methylquinoline which they deduced to be the 3-bromo derivative. [c] The structure of this compound was not proved. It was prepared from *p*-nitroaniline and the tribromoaldehyde. [d] This compound was prepared by two methods: in 15% yield by the reaction of the tribromoaldehyde with anthranilic acid; in 66% yield by the reaction of the tribromoaldehyde with *m*-bromoanthranilic acid. In each case the reactants were mixed and heated in the absence of solvent until reaction occurred. Acetic acid then was added and the solution was boiled for three hours and worked up as before. [e] G. Bendy, C. C. J. Culvenor, L. J. Goldsworthy, K. S. Kirby and R. Robinson, *J. Chem. Soc.*, 1130 (1950), report m.p. 157–158°.

Isolation of Intermediates.—2-Nitro-4-methylaniline (0.02 mole) was stirred with 2,2,3-tribromopropanal (0.01–0.02 mole) until a thick paste was obtained. This was warmed on a steam-bath for 30 minutes and the resulting hard mass crystallized from alcohol. The bright orange crystals melted 165–160°. The yield was 25%.

Anal. Calcd. for $C_{17}H_{16}Br_2N_4O_4$: C, 40.82; H, 3.22. Found: C, 41.11, 41.10; H, 3.43, 3.45.

When this compound was warmed in acetic acid, a mixture of 3-bromo-6-methyl-8-nitroquinoline and its hydrobromide precipitated. Stirring with ammonia and crystallizing from alcohol gave pure 3-bromo-6-methyl-8-nitroquinoline.

The intermediate was formed in the same way with *o*-nitroaniline. In this case the product was bright yellow, m.p. 180–181°. The yield was 12%.

Anal. Calcd. for $C_{15}H_{12}Br_2N_4O_4$: C, 38.16; H, 2.56. Found: C, 38.05, 38.46; H, 2.98, 2.71.

Warming this compound in acetic acid and working up in the usual way yielded 3-bromo-8-nitroquinoline, m.p. 120–122°, identified by mixed m.p. with authentic sample.[4]

(3) Analyses by Oakwold Laboratories, Alexandria, Va.

(4) C. R. Hauser, M. S. Bloom, A. S. Breslow, J. T. Adams, A. T. Amore and M. J. Weiss, THIS JOURNAL, **68**, 1544 (1946).

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The Oxidation of Stilbestrol in Alkali[1]

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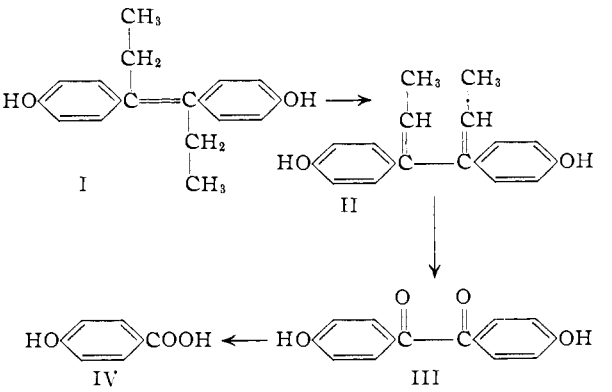
Aqueous neutral or alkaline solutions of diethylstilbestrol kept for two weeks at room temperature or in the cold become yellow in color and lose their estrogenic activity.[2] This instability is greater in the presence of air or oxidizing agents.[3] The isolation of the alkaline oxidation products of stilbestrol was undertaken by us after the crude reaction mixture was found to be effective in causing the release of pituitary gonadotropins.[4] The biological properties of several of the isolated oxidation products have been reported.[4b]

When "concentrated" solutions of stilbestrol (5 mg./cc.) in 0.02 *N* NaOH were oxygenated and allowed to stand at room temperature or refluxed, isodienestrol[5] was isolated in 17–33% yield; 10–27% of the stilbestrol was recovered unchanged. The oxidation of diethylstilbestrol (I) to isodienestrol (II) by the removal of two hydrogens indicates the initial mechanism whereby the estrogenic activity of alkaline stilbestrol solutions is lost on stand-



(1) This investigation was supported in part by research grant C-2161 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) B. Zondek and F. Sulman, *Endocrinol.*, **33**, 204 (1943).

(3) (a) A. E. Wilder-Smith and P. C. Williams, *Nature*, **156**, 718 (1945); (b) A. E. Wilder-Smith and P. C. Williams, *J. Endocrinol.*, **5**, 152 (1947); (c) F. L. Warren, F. Goulden and A. M. Robinson, *Biochem. J.*, **42**, 151 (1948).

(4) (a) O. W. Smith, private communication, 1945; (b) O. W. Smith and R. E. Vanderlinde, *Endocrinol.*, **49**, 742 (1951).

(5) (a) G. J. Hobday and W. F. Short, *J. Chem. Soc.*, 609 (1943); British Patent 566,581 (Jan. 4, 1945); (b) H. v. Euler and E. Adler, *The Svedburg (Mem. Vol.)*, 246 (1944); (c) J. F. Lane and L. Spialter, THIS JOURNAL, **73**, 4408 (1951).

(6) R. E. Vanderlinde and W. W. Westerfeld, *Federation Proc.*, **8**, 262 (1949).

ing since isodienestrol is only $^1/_{600}$th as active estrogenically as stilbestrol.[4b,6]

Further degradation of the stilbestrol molecule occurred when more dilute solutions were used (3 or 30 μg./cc. of 0.001 or 0.01 *N* NaOH, respectively). No isodienestrol was recovered under these conditions, but small amounts of three additional oxidation products were isolated by acetylation of the crude extract and chromatographic separation. Free 4,4′-dihydroxybenzil (III) was isolated in 1.03 and 1.75% yield from two of eight inactivated stilbestrol preparations so treated, and the diacetate of dihydroxybenzil was isolated in trace amounts and in 1.1% yield from two other such preparations. The 4,4′-dihydroxybenzil was apparently formed by oxidation at the double bonds of the intermediate isodienestrol (II). The infrared spectrogram of 4,4′-dihydroxybenzil was unusual in that it failed to show any absorption in the range of normal carbonyl groups[7] but extreme absorption between 6 and 7 μ indicating that it probably exists as the mono- or dienol-quinone. On the other hand, the infrared spectrogram of the diacetate of 4,4′-dihydroxybenzil markedly resembles that of *p*-acetoxybenzoic acid suggesting that it is the ester of the phenolic rather than the enolic hydroxyl groups. The 4,4′-dihydroxybenzil showed a single ultraviolet absorption maximum at 300 mμ, whereas the diacetate was shifted to 267.5 mμ (see Table I).

p-Acetoxybenzoic acid was isolated in 1.6 to 6.5% yield from four out of eight preparations of oxidized stilbestrol, and the free *p*-hydroxybenzoic acid (IV) was recovered from one batch. The latter would appear to represent the end product of at least one possible pathway of oxidation of stilbestrol *via* isodienestrol (II) and dihydroxybenzil (III).

An unidentified product also was isolated from the acetylated mixture as white needles melting at 157° (previously[4b] and hereafter referred to as the "157° acetate"). It was isolated in 0–2.90% yield from stilbestrol preparations of 3 μg./cc. of 0.001 *N* NaOH or 30 μg./cc. of 0.001 *N* NaOH which had stood for two weeks at room temperature. Analysis provided a molecular formula of $C_{20}H_{18}O_7$. The ultraviolet absorption spectrum had a single maximum at 250 mμ (see Table I). Its infrared spectrogram showed the presence of an ester carbonyl at 5.71 mμ, possibly an aliphatic carbonyl at 5.82 mμ and an aryl carbonyl at 5.88 mμ. One attempt to saponify the 157° acetate and recover the free form in a satisfactory yield was unsuccessful. This compound is of special interest[4b] because of its biological properties, but its exact structure has not been established.

Certain related synthetic estrogens were oxidized under similar conditions (30 μg./cc. of 0.001 *N* NaOH at room temperature for 2 weeks), worked up by acetylation and chromatographic separation, and found to yield some of the same oxidation products. Dienestrol gave 1.9 and 2.9% of *p*-acetoxybenzoic acid and 0.45% of the 157° acetate. Isodienestrol yielded 1.7% of the free dihydroxybenzil as the only identifiable oxidation product. Indenestrol "A," the product formed by the cyclization of dienestrol or isodienestrol with acid,[8] yielded 0.7% of *p*-acetoxybenzoic acid as the only identifiable oxidation product.

Several other inactivation procedures were attempted in order to increase the yield of oxidation products. An Oppenauer oxidation of stilbestrol using diacetyl as a hydrogen acceptor was found to yield 6.0% isodienestrol. Dienestrol was recovered unchanged following treatment with 2% hydrogen peroxide at room temperature for 108 hours in 0.8 *N* NaOH, and was converted by peracetic acid to a black resin from which no identifiable products could be isolated. Treatment of the diacetate of dienestrol with peracetic acid resulted in a 4.4% yield of a crystalline product which contained two additional acetate groups.

Other published procedures related to this problem include the following: The lead tetraacetate oxidation of stilbestrol in ether–chloroform yields isodienestrol.[5b] Perbenzoic acid reacts with stilbestrol to form epoxystilbestrol which on heating rearranges to form a pinacolone, 3,3-bis-(*p*-hydroxyphenyl)-4-hexanone.[9] Permanganate oxidation or ozonolysis of the dimethyl ethers of isodienestrol or dienestrol yields only small amounts of anisil and some acetaldehyde.[5a] Dienestrol in alcoholic KOH at 220° is rearranged to its stereoisomer, isodienestrol without any significant oxidation.[5a]

Experimental

Oxidation of Stilbestrol (5 mg./ml.).[10]—Stilbestrol[11] (3.5 g.) was dissolved in 1850 ml. of 0.02 *N* NaOH and oxygen was bubbled through the solution for a few minutes every other day for two weeks. Acidification to *p*H 3 with concentrated HCl, ether extraction, and removal of the ether yielded a semi-crystalline residue. When washed with 5 cc. of cold benzene, 1.9 g. of crude crystals remained. Crystallization from benzene gave 1.7 g. of light yellow isodienestrol crystals, m.p. 181–183°. This material was recrystallized 12 times by dissolving it in ethanol and gradually replacing the alcohol with benzene as the former was boiled off. Light yellow crystals, 0.078 g., m.p. 183–184°, were obtained, and when mixed with authentic isodienestrol[12] of m.p. 182–183° gave a mixed m.p. of 182–184°.

Anal. Calcd. for $C_{18}H_{18}O_2$: C, 81.17; H, 6.77. Found[13] (1): C, 80.37; H, 7.13. Found (2): C, 80.40; H, 6.92.

Acetylation of 0.370 g. of the isolated isodienestrol with acetic anhydride–pyridine, and recrystallization from ethanol gave 0.300 g. of isodienestrol diacetate, m.p. 146–147°.[12]

The initial benzene leachings from the semi-crystalline residue yielded 0.37 g. of unchanged stilbestrol and 0.92 g. of tars from which no crystalline material could be obtained. Isodienestrol was isolated in 16–28% yield in 2 of 3 other similar experiments carried out at room temperature or at 37°. It was obtained in 20% yield when the stilbestrol solution was refluxed for 8 hr. with a stream of air

(7) F. A. Miller in "Organic Chemistry, an Advanced Treatise," Volume III, H. Gilman, Editor, John Wiley and Sons, Inc., New York, N. Y., 1953, pp. 146–147.

(8) (a) E. Adler and B. Hagglund, *Arkiv. Kemi Mineral. Geol.*, **19A**, No. 23, 1 (1945); (b) W. Hausmann and A. E. Wilder-Smith, *Nature*, **161**, 892 (1948); (c) W. Hausmann and A. E. Wilder-Smith, *J. Chem. Soc.*, 1030 (1949).

(9) F. Wessely, E. Kerschbaum, E. A. Kleedorfer, R. Prillinger and E. Zajic, *Monatsh.*, **73**, 127 (1940).

(10) All melting points in this section were made in capillary tubes and are uncorrected.

(11) Courtesy of Ayerst, McKenna and Harrison, Rouses Point, N. Y.

(12) Prepared according to reference 5b and a sample obtained from C. W. Sondern, White Laboratories, Newark, N. J.

(13) Analyses by Huffman Microanalytical Laboratory, Wheatridge, Colo., and by Samuel P. Sadtler and Son, Inc., Philadelphia, Pa.

continuously passing into the solution, and then aerated at room temperature for 24 hours.

Oxidation of Stilbestrol (3 μg./ml.).[14]—Stilbestrol (2.4 g.) was dissolved in 800 ml. of 1 *N* NaOH, and 20-ml. aliquots were added to each of 40 five-gallon bottles of distilled water (3 μg./ml. of 0.001 *N* NaOH). Oxygen was passed daily into each bottle for approximately two minutes. After two weeks the yellow solutions were acidified and extracted as previously described.

The combined ether extract contained 1.834 g. of crude oils, which were dissolved in 50 ml. of ethanol–benzene (4:46) and poured onto a column of 15 g. of silicic acid–Celite (2:1) washed previously with benzene.[15]

ELUTION WITH BENZENE AND ETHER

Fraction	Cc.	Eluent	Wt., g.
A	200	Benzene	0.916
B	200	Ether–benzene (1:31)	.610
C	300	Ether	.232
D	50	95% EtOH	.029
			1.787

Three major oily fractions separated which could not be crystallized. Each fraction therefore was acetylated on a steam-bath for 45 minutes with 5 times its weight of pyridine–acetic anhydride (3:1). The oily acetates were extracted with ether, washed with dilute HCl and H_2O, dried over anhydrous Na_2SO_4, and the ether removed by distillation.

The acetylated fraction A was taken up in 15 cc. of benzene and chromatographed on a column of 10 g. of silicic acid–Celite (2:1) as follows

Fraction	Cc.	Eluent	Weight, g.
A-1	100	Benzene	0.196
A-2	100	Ether–benzene (5:95)	.490
A-3	150	Ether–benzene (25:75)	.163
			0.849

Fraction A-2 after several recrystallizations from ethanol yielded 43 mg. of white crystals, m.p. 155–157°. An additional 27 mg. was obtained from the acetylated fraction B. No crystalline material was obtained from fractions C, A-1 or A-3. Other similar preparations of inactivated stilbestrol carried through approximately the same procedure yielded 6 mg. of the 157° acetate plus 3 mg. of the diacetate of dihydroxybenzil, or 32 mg. of the 157° acetate plus 96 mg. of *p*-acetoxybenzoic acid.

157° Acetate: white crystals from ethanol; m.p. 157–158°.

Anal. Calcd. for $C_{20}H_{18}O_7$: C, 64.87; H, 4.86; mol. wt., 370. Found (1): C, 64.92; H, 4.88; mol. wt., 385. Found (2): C, 65.05; H, 4.75; mol. wt., 363.

Saponification.—Thirty-two mg. of the 157° acetate was saponified with 6 cc. of 10% alcoholic NaOH at room temperature. After 16 hours the ethanol was evaporated on a water-bath *in vacuo* and 20 cc. of water was added. The solution was acidified to *p*H 3, and extracted with ether. Crystallization of the ether-soluble residue from water, yielded only 2.4 mg. of white crystals m.p. 242–243° whose ultraviolet absorption maximum is recorded in Table I, along with the other compounds in this series.

Three similar preparations of 2.4 g. of stilbestrol, but 10 times as concentrated, *i.e.*, 30 μg./ml. of 0.01 *N* NaOH, yielded 42 mg. of dihydroxybenzil and 40 mg. of *p*-acetoxybenzoic acid, or 27.2 mg. of the diacetate of dihydroxybenzil and 50.7 mg. of *p*-acetoxybenzoic acid, or 155.0 mg. of *p*-acetoxybenzoic and 8.9 mg. of the 157° acetate.

(14) All melting points in this and subsequent sections were taken on a Fisher-Johns melting point block and are uncorrected.

(15) At the suggestion of Dr. C. W. Sondern, White Laboratories, Newark, N. J., the method later described by Lane and Spialter[16] was utilized with modifications. The solvents were pulled through by vacuum. This method, while not entirely satisfactory, aided in purifying and partially separating some of the oxidation products.

TABLE I

ULTRAVIOLET ABSORPTION MAXIMA[a]

Compound	Free phenol Max.	Free phenol O.D.	Acetate Max.	Acetate O.D.
Stilbestrol (Diethyl)	237.5	0.910	220	1.020
Dienestrol	230	1.800	225	1.755
Isodienestrol	265	1.315	257.5	1.345
Indenestrol "A"	310	1.365	302.5	1.125
4,4′-Dihydroxybenzil	300	1.400	267.5	1.280
p-Hydroxybenzoic acid	255	1.510	233	1.270
157° Acetate	280	1.200	250	1.295

[a] Each compound was dissolved in 95% ethanol at a concentration of 13.4 μg./cc. for the free phenol and 17.6 μg./cc. for the acetates (0.00005 *M* for diethylstilbestrol and its diacetate). Optical density readings were taken with a Beckman DU spectrophotometer every 10 mμ from 370 to 230 mμ. The ultraviolet spectra of all known compounds isolated from the inactivation reactions were identical with those of authentic samples.

4,4′-Dihydroxybenzil: yellow needles from water; m.p. 252°.

Anal. Calcd. for $C_{14}H_{10}O_4$: C, 69.42; H, 4.13; mol. wt., 242. Found (1): C, 68.46; H, 4.45; mol. wt., 245. Found (2): C, 69.04; H, 4.57.

The isolated compound gave identical ultraviolet and infrared spectra with an authentic sample of dihydroxybenzil prepared by the demethylation of anisil.[16]

Diacetate of 4,4′-dihydroxybenzil: light yellow crystals from ethyl alcohol; m.p. 87°.

Anal. Calcd. for $C_{18}H_{14}O_6$: C, 66.26; H, 4.29; mol. wt., 326; acetyl (two groups), 30.08. Found (1): C, 66.74; H, 4.75; mol. wt., 282; acetyl, 29.92. Found (2): C, 67.21; H, 5.15.

Acetylation of dihydroxybenzil obtained from demethylated anisil[16] yielded this same diacetate, m.p. 86° (mixed m.p. 86–87°).

Anal. Calcd. for $C_{18}H_{14}O_6$: C, 66.26; H, 4.29; mol. wt., 326. Found (1): C, 66.57; H, 4.45; mol. wt., 281. Found (2): C, 66.51; H, 4.39; mol. wt., 290.

***p*-Acetoxybenzoic acid**: white needles from benzene or xylene; m.p. 190°.

Anal. Calcd. for $C_9H_8O_4$: C, 60.00; H, 4.44; mol. wt., 180; carboxyl, 25.11; acetyl, 23.88. Found (1): C, 60.36; H, 4.38; mol. wt., 183; carboxyl, 25.11; acetyl, 23.75. Found (2): C, 59.91; H, 4.53.

Peracetic Acid Plus Dienestrol Diacetate.—Dienestrol diacetate (2.4 g.), m.p. 117–119°, and 0.03 g. of *p*-toluenesulfonic acid were added to 30 ml. of peracetic acid (5.66 ml. of 40% peracetic acid diluted with acetic acid to 30 ml.) and the reaction mixture was allowed to stand in the dark at 4° for 110 hours. The reaction mixture was diluted with water, and the precipitated oil was extracted with ether. The ether extract was dried over anhydrous Na_2SO_4, and evaporated to yield 2.323 g. of oil. Crystallization from ethanol yielded white crystals, m.p. 205–210°. On further recrystallization 0.107 g. was obtained, m.p. 236–239°. A mixed melting point with free dienestrol (m.p. 233°) was 195–200°.

Anal. Calcd. for $C_{26}H_{30}O_8$: C, 66.38; H, 6.38; mol. wt., 470. Found: C, 66.49; H, 6.32; mol. wt., 446.

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