Estrogenic Action and Molecular Structure

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This paper presents a hypothesis regarding an optimum molecular thickness as a structural requirement for the development of a strong estrogenic activity.

The preparation of various estrogenic oxygen and sulfur analogs of hexestrol or its dimethyl ether, reported from this laboratory,(1),(2),(3) has demonstrated that the similarity in the structural features, consisting of the shapes of molecular skeletons and the nature and the positions of functional groups, may cause similar physiological properties. It was shown by X-ray crystallography(4) that the stereochemical arrangement of the atoms in hexestrol is very closely related to the stereochemical form of the natural estrogens. On the other hand, diethylstilbestrol would possess a planar structure, significantly thinner than those of hexestrol and natural estrogens, if its two ethyl groups did not exert a steric interference upon the benzene nuclei and thus the whole system were completely conjugated.

F. W. Schueler(5) suggested that a given substance may be estrogenic if it consists of a rather large, rigid, and inert molecular structure with two active hydrogen bond-forming groups located at an optimum distance of 8.55 A. from one another. E. R. Clark(6) modified Schueler's hypothesis by introducing the conception of "anchoring" in order to explain the estrogenic activity of non-rigid molecules. There are, however, many substances which have little activity even though they apparently satisfy Schueler's requirements, as exemplified by 4, 4′-dihydroxystilbene and 4, 4′-dihydroxy-2, 2′-diethylstilbene. Schueler seems to find an example to meet his hypothesis in *trans-p, p′*-dihydroxyazobenzene which is said to show definite estrogenic activity in dosages of from 10 to 15 mg. when injected subcutaneously in

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(1) Yoshiyuki Urushibara and Michinori Ōki, this Bulletin, **23**, 35 (1950).

(2) Y. Urushibara and Takeyoshi Takahashi, *ibid.*, **23**, 53 (1950).

(3) Y. Urushibara, M. Ōki, and Reiji Ikeda, *ibid.*, **25**, 66 (1952).

(4) C. H. Carlisle and D. Crowfoot, *J. Chem. Soc.*, **1941**, 6.

(5) F. W. Schueler, *Science*, **103**, 221 (1946).

(6) E. R. Clark, *J. Chem. Soc.*, **1950**, 3397.

rats. But it should be considered that this substance is rather weak in spite of its fulfilment of the requirements, because Schueler gives examples of substances more active but not meeting his requirements.

The present authors measured the absorption spectra and the extinction coefficients of 4,4′-dimethoxystilbene, $CH_3OC_6H_4CH=CHC_6H_4OCH_3$, and its α,α'-dialkyl homologs. They are shown in the accompanying figure with the absorption curve of *p*-cresol methyl ether. The unsubstituted dimethoxystilbene shows a maximum at the longest wave length with the greatest molecular extinction coefficient. The dimethyl homolog gives a maximum at a shorter wave length and a smaller molecular extinction coefficient, and, as a larger alkyl group is substituted, the absorption curve is displaced further toward a shorter wave length and a smaller molecular extinction coefficient, until, presumably, that of *p*-cresol methyl ether is reached at the extremity.

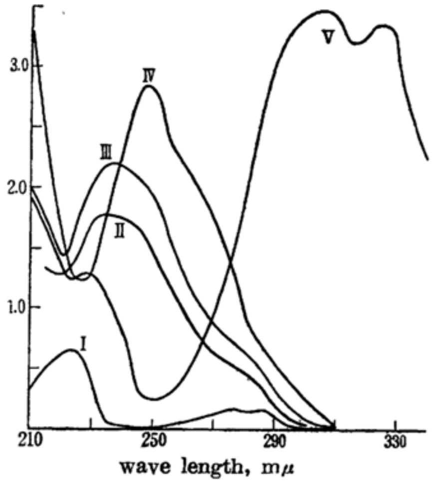

Fig. 1.—Ultraviolet absorption spectra of *p*-cresol methyl ether (I), 4,4′-dimethoxy-α,α′-dipropylstilbene (II), 4,4′-dimethoxy-α,α′-diethylstilbene (diethylstilbestrol dimethyl ether)(III), 4,4′-dimethoxy-α,α′-dimethylstilbene (IV), and 4,4′-dimethoxystilbene (V) in 95% ethanol.

The displacement of the absorption curves from that of unsubstituted dimethoxystilbene can be interpreted as showing the extent of decrease in the degree of conjugation, which in turn can be attributed to a deviation of the benzene nuclei from the coplanar structure due to a rotation about the 1,4- and 1′,4′-axes enforced by the steric interference of the alkyl groups at the α,α'-positions. Then, it will be proved that diethylstilbestrol does not possess a planar structure, and it is natural to suppose that it will possess an optimum molecular thickness (the dimension perpendicular to the plane of the ordinary projection formula) as the most potent member of its series. Since steric interference is mutual, the two ethyl groups may not be able to rotate freely or independently of the benzene nuclei. Thus, the ethyl groups will be arranged in a manner coördinated with the rotated benzene nuclei, and it can be said that the ethyl groups impart the most appropriate structural features to the molecule of diethylstilbestrol by their own bulks on one hand, and, on the other, by exerting a steric interference on the benzene nuclei to cause their deviation from a coplanar conjugation.

It must be noticed that the molecular thickness is of course correlated with the width for a given structural formula and the width may possibly have no less importance on the physiological properties, but the thickness is mentioned here to express explicitly the significance of the definite deviation from a planar structure and to put a primary stress on the nature of the flatness of the molecule, because the natural estrogens and consequently hexestrol are known to possess rather flat structures.

Examination of scale models also reveals that diethylstilbestrol is sterically hindered while the 2,2′-diethyl isomeride is not hindered. There exists no steric interference in 4,4′-dihydroxystilbene as is shown by the absorption spectrum of its dimethyl ether and also in *trans*-*p*,*p*′-dihydroxyazobenzene as is understood from its constitution. The small activity of these substances capable of taking the most conjugated planar structures can be explained by considering that their structures are too thin to develop a strong activity although they are favored with the location of the two hydrogen bond-forming groups at the optimum distance.

In a previous paper⁽³⁾ the authors and R. Ikeda described the synthesis of 1,2-bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethylene, $CH_3OC_6H_4C(SCH_3)=C(SCH_3)C_6H_4OCH_3$, which was active in the dosage of 10 γ in mice. It gave an absorption spectrum indicating that the steric interference of the methylthio groups corresponds nearly to that of the propyl groups in 4,4′-dimethoxy-α,α'-dipropylstilbene.

In this connection, it may be pointed out that it is dangerous to attempt a determination of *cis*- and *trans*-forms of stilbene derivatives from absorption spectra.⁽⁷⁾

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(7) D. H. Hey and O. C. Musgrave, *J. Chem. Soc.*, **1949**, 3156.

Experimental Part

The absorption spectra were recorded with a Beckman Model DU photoelectric quarz spectrophotometer. The length of solutions was 5 mm. The concentrations of the solutions are shown below:

p-Cresol methyl ether (b. p. 176°)	17.75×10^{-5} mol./l.
4, 4′-Dimethoxystilbene (m. p. 212—213°)	5.25 ″
4, 4′-Dimethoxy-α, α′-dimethylstilbene (m. p. 140.5—142°)	5.26 ″
4, 4′-Dimethoxy-α, α′-diethylstilbene (Diethylstilbestrol dimethyl ether) (m. p. 121.5—123°)	2.80 ″
4, 4′-Dimethoxy-α, α′-dipropylstilbene (m. p. 111—113°)	6.45 ″

4, 4′-Dimethoxystilbene, $CH_3OC_6H_4CH=CH\text{-}C_6H_4OCH_3$. Prepared by the methylation of 4, 4′-dihydroxystilbene.

4, 4′-Dimethoxy-α, α′-diethylstilbene (Diethylstilbestrol dimethyl ether), $CH_3OC_6H_4C(C_2H_5)=C(C_2H_5)C_6H_4OCH_3$. Synthesized by the method of Vargha and Kovács.(8)

p-Methoxyacetophenone-hydrazone, $CH_3OC_6H_4\text{-}C(CH_3)=NNH_2$. p-Methoxyacetophenone (5.0 g.), prepared from anisole and acetic anhydride,(9) and 72% hydrazine hydrate (3.3 g.) were dissolved in warm alcohol (5 cc.) and the solution was heated on a water bath for 5 hours. After cooling, the formed crystalline product was collected and recrystallized from alcohol. Colorless needles (Yield 5.0 g.), m. p. 118.5—120°. Found: N, 17.03%. Calculated for $C_9H_{12}ON_2$: N, 17.06%.

4, 4′-Dimethoxy-α, α′-dimethylstilbene, $CH_3\text{-}OC_6H_4C(CH_3)=C(CH_3)C_6H_4OCH_3$. The above hydrazone (4.0 g.) and yellow mercuric oxide (8.0 g.) suspended in petroleum ether (b. p. 40—60°) (100 cc.) were shaken at 23—24° for 16 hours. The resulting red solution was filtered and sulfur dioxide was passed vigorously into the solution under cooling, until the color disappeared and instantly bubbles were generated. Petroleum ether was evaporated and the residue was heated to 150° to complete decomposition. The substance, dissolved in benzene, was adsorbed chromatographically on alumina and recrystallized from alcohol. Colorless plates (Yield 0.6 g.), m. p. 140.5—142°. Found: C, 80.45; H, 7.59%. Calculated for $C_{16}H_{20}O_2$: C, 80.56; H, 7.51%. Dodds *et al.*(10) give m. p. 127—128°.

4, 4′-Dimethoxy-α,α′-dipropylstilbene, $CH_3O\text{-}C_6H_4C(C_3H_7)=C(C_3H_7)C_6H_4OCH_3$. p-Methoxybutyrophenone, prepared from anisole and butyryl chloride,(11) gave a difficultly crystallizable hydrazone. The crude hydrazone (5 g.) and yellow mercuric oxide (10 g.) were treated in the same way as described above. Recrystallized from alcohol, the product was obtained in colorless rhombic prisms (Yield 0.3 g.), m. p. 111—113°. Found: C, 81.77; H, 8.90%. Calculated for $C_{22}H_{28}O_2$: C, 81.44; H, 8.70%. The minimum active dose in mice was found 100 γ. Dodds *et al.*(10) give a boiling point 178—181°/0.8 mm. but no melting point. They record a minimum active dose of 100 γ in rats for the corresponding free phenol.

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(8) F. L. Vargha and E. Kovács, *Ber.*, **75**, 794 (1942).

(9) J. B. Conant and C. R. Kinnet, "Org. Syntheses" Coll. Vol., I, p. 105.

(10) E. C. Dodds, L. Golberg, W. Lawson, and R. Robinson, *Proc. Roy. Soc.*, B, **127**, 140 (1939).

(11) P. M. Baranger, *Bull. soc. chim. France*, [4], **49**, 1214 (1931).