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MOLECULAR STRUCTURE AND ESTROGENIC ACTIVITY—I

UNSYMMETRICAL DIPHENYLETHYLENES AND TRIPHENYLETHYLENES

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Abstract—Variation in the planarity of estrogenic substituted ethylenes has been studied by means of U.V. absorption.

1. In the *unsym*-dianisylethylenes, β-substituents increase the steric hindrance. Symmetrical and unsymmetrical twisting of the rings is discussed.

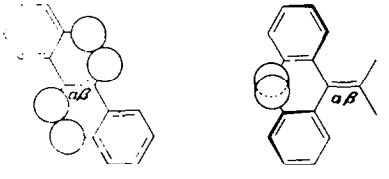
2. Preceding results are extended to trianisylethylene in which different structures are considered: partially non-planar anethole, *cis*-, and *trans*-di-*p*-methoxystilbene. Introduction of a β-chloro atom causes the twisting of all aromatic groups out of the double bond plane. Thus π–π conjugation is considerably reduced giving rise to a new band which is practically not affected by the presence of *para*-methoxy substituents. Increase of estrogenic activity is correlated with this structural modification.

3. Non-planarity alone of a single ring is not sufficient to induce high estrogenic activity, the second ring in the α-position is itself necessary for high activity and the *unsym*-diphenylethylene structure seems to play an important role in the activity of the halogenated-triphenylethylenes. This may explain several findings reported in the literature.

4. The relation between estrogenic activity and structure in these substituted ethylenic compounds should be discussed not only in terms of spatial structure but also as in terms of electron distribution.

THE first workers to indicate any relation between non-planar structure and estrogenic activity were Lewis and Calvin.[1] Jones[2] classified *unsym*-diphenylethylenes, stilbene, substituted stilbenes, and triphenylethylene into two groups, governed by the similarity of the U.V. absorption spectra to those of *trans*-stilbene and styrene. This study is the basis of several subsequent works, and may be summarized as follows.

In the first group (Fig. 1) the maximum at 295 mμ is thought to represent the absorption of a conjugated system along the whole length of the molecule, in which all the carbon atoms are situated along the plane determined by the central double



I II

bond; the resonance energy is maximum. To such a system belong both *trans*-stilbene (I)* and triphenylethylene, which show very similar absorption in the 295

* Molecular dimensions and bond distances are approximate and are drawn only in order to visualize steric effects.

[1] G. N. Lewis and M. Calvin, *Chem. Rev.* **25**, 302 (1939).
[2] R. N. Jones, *J. Amer. Chem. Soc.* **65**, 1818 (1943).

mμ region. In the second group, *unsym*-diphenylethylene (II) and α-β-substituted-ethylenes shows a maximum in the 240 mμ region as does styrene (Fig. 1);

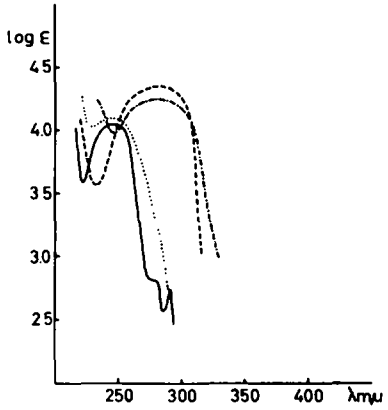


FIG. 1.—styrene (ethanol); . . . . α-β-dimethyl-*trans*-stilbene (ethanol); – – – *trans*-stilbene (chloroform); –.–.–. triphenylethylene (chloroform). (After Jones[3])

Jones has given an empirical interpretation of this result by assuming that the hydrogen atoms situated in the *ortho* position on the adjacent rings (II) are too close to each other, and the hindered structure so obtained is thought to force one of the rings out of the plane of the double bond. In such a case this ring is prevented from being conjugated with the rest of the molecule, and this could explain the fact that the spectrum of the molecule in the region studied is the same as if the ring had been replaced by a hydrogen atom.

Several subsequent studies of the relation between U.V. absorption and estrogenic activity have shown that for stilbene derivatives the presence of such a non-planar structure is significant for estrogenic activity. Jeffrey *et al.*[3] used physical techniques to prove the *trans-trans* structure of dienestrol (α-dienestrol) and *trans*-diethylstilbestrol. In a series of papers Ōki,[4] studied diethylstilbestrol and analogues. Recently Grundy[5] has made a general review of this problem, since then Ōki[6] has published several interesting studies on the relation between activity and non-planarity in the biphenyl series.

The present paper reports an investigation along the line of the works just mentioned, and its particular purpose is to examine by means of U.V. absorption spectra, the non-planarity in the substituted *unsym*-dianisylethylenes and trianisylethylenes in relation to their estrogenic activity. The importance of the non-planar *unsym*-dianisylethylene group for strong estrogenic activity is discussed.

In connexion with the general problem to which this paper relates, it is appropiate to emphasize here two points which are important for the following discussion and which previous workers have often neglected to mention explicitly:

The relation, between changes in planarity and variations in position and intensity of the bands here considered, is not exact, due to the fact that changes in U.V.

[3] G. A. Jeffrey, H. O. Koch and S. C. Nyburg, *J. Chem. Soc.* 1118 (1948).
[4] M. Ōki, *Bull. Chem. Soc. Japan* **26**, 37, 161, 331 (1953); M. Ōki and Y. Urushibara, *Ibid.* **25**, 109 (1952).
[5] J. Grundy, *Chem. Rev.* **57**, 281 (1957).
[6] M. Ōki, *Bull. Chem. Soc. Japan* **30**, 859 (1957).

absorption reflect differences in conjugation of the various groups and not directly the spatial structure. We shall introduce here a restriction, namely the assumption that the different substituents which are introduced into the molecule do not influence strongly, by their own electronic behaviour, the total electronic state of the molecule. With this restriction, position and intensity of the "K" band can be correlated to the planarity of the molecule.

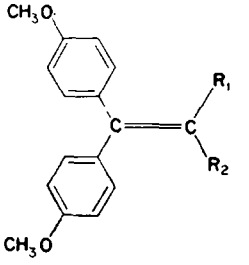
para-Methoxy derivatives have been used in this study because hydroxylated compounds were not available in every case. This has the disadvantage of introducing one more metabolic step between the injection of the molecule and the action on the biological receptor, if, as it is believed,[5] the molecule has to be demethylated before acting. In trying to establish differences in "inherent" or "true" estrogenic potency between different members of a series is has to be assumed that the splitting of the methyl ether occurs with identical speed for every term of the series and also the absorption and elimination of the estrogen from the body. Such factors are until now unknown and make difficult any attempt to correlate variation in molecular structure with "inherent" or "true" estrogenic activity.

U.V. absorption and estrogenic activity in substituted unsym-*dianisylethylenes*

The U.V. absorption spectra of 1,1-di-*p*-hydroxyphenyl-2,2-diethyl-ethylene ($\alpha\alpha$DE) and its dimethyl ether (III) have been recorded in a preceding report.[7]* They are characterized by a great similarity to the spectra of α-dienestrol and also *trans*-diethylstilbestrol. The position and intensity of the short wavelength band are nearly identical for the three compounds.

Braude,[8] in a study of the 220 mμ band in diphenylmethane, and dibenzyl, has discussed the possibility of interaction of phenyl chromophores through one or two methylene groups. He has also suggested a similar interpretation for the 226 mμ band of the *trans*-stilbene and for the 228 mμ band of the 4,4'-dihydroxy-*trans*-stilbene. All those different bands called E' bands are very similar and are attributed to a partial dibenzyl chromophore.†

The main absorption in di-substituted stilbene is at 240 mμ and may be regarded as a slightly displaced E' band resulting from the partial dibenzyl chromophore,



* It has been found that 1,1-di-*o*-hydroxyphenyl-2,2-diethyl-ethylene described as a new compound has previously been obtained by Z. Földi, and I. Demjén, *Ber. Dtsch. Chem. Ges.* **74**, 930 (1941).

† i.e. a possible interaction of the π electrons of the phenyl rings through the σ electrons of the ethane or ethylene bridge.[8] Similar interactions of a phenyl chromophore with a carbonyl group through a saturated carbon have also been described by W. D. Kumler, L. A. Strait and E. L. Alpen, *J. Amer. Chem. Soc.* **72**, 1463 (1950), who give several other references.

[7] J. F. Miquel, *Acta Chem. Scand.* **12**, 274 (1958).

[8] E. A. Braude, *J. Chem. Soc.* 1902 (1949).

since the band characteristic of the π–π conjugation has now disappeared because of the non-planarity of the system.

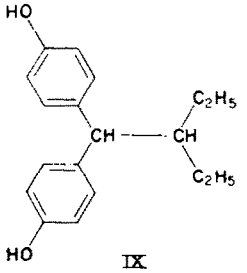
The present result could lend further support to this view, it appears from the findings presented in Table 1 that the 241 mμ band in compound III ($\alpha\alpha$DE dimethyl

TABLE 1

Substituents			First band (E′)		Shoulder or second band		Effective dose in mice in micrograms	Mp.	Rf.
No.	R_1	R_2	λ_M	log ε_M	λ_M	log ε_M	50% estrus		
III	C_2H_5	C_2H_5	241	4·29	275–290	3·70	4000	91°	7
IV	H	H	248	4·40	255–270	4·25	Inactive 20 mg	140°	9
V	H	CH_3	245	4·32	255–270	4·25	Inactive 20 mg	99°	9
VI	CH_3	CH_3	241	4·28	...	...	10,000	65°	10
VII	H	C_2H_5	244	4·41	260–270	4·25	Inactive 20 mg	98°	11
VIII	CH_3	C_2H_5	240	4·30	...	...	10,000	49°	12
IX	Ethane deriv		235	4·22	276	3·61	7500	168°	9
Transdiethylstilbestrol			239	4·25	270–280	3·85	0·1	171°	8
Dienestrol			229	4·43	270–280	3·70	0·1	230°	8

ether) and homologues could be assimilated to a E' band due to their position and to the fact that, as in the substituted stilbenes, the band is little affected by the presence of hydroxy or alkoxyl substituents. This is not the place to discuss the meanings and the limits of the explanation proposed by Braude for the nature of the 220 mμ and the assimilation of the stilbene's 240 mμ band to such a system but his appealing hypotheses will be accepted in this paper and the short wavelength band of the compound studied called E' following his terminology.

Starting from the *unsym*-anisylethylene (IV in Table 1) there is a slight but definite hypsochromic shift of the E' band, following the increase in steric hindrance obtained by the introduction of substituents on R_1 and R_2. The reduction in the intensity is parallel. In the case of mono-substitution, the maximum for the methyl derivative is at a shorter wavelength, the intensity is lower, than in the case of the ethyl analogue. The U.V. maxima of *trans*-diethylstilbestrol-dimethylether and α-dienestrol are listed in Table 1 for comparison, and also the ethane derivative of $\alpha\alpha$DE (IX), in which



IX

[9] L. Gatterman, *Ber.* **22**, 1130 (1889).
[10] C. Mentzer and N. Dat-Xuong, *Bull. Soc. Chim. Fr.* 885 (1947).
[11] P. Weill, *Bull. Soc. Chim. Fr.* 1811 (1931).
[12] J. F. Miquel (to be published).

band E' is slightly shifted to shorter wavelengths with a decrease in intensity, and the characteristic shoulder at 270–280 mμ is replaced by a band at 276 mμ. In Table 1 are also listed estrogenic activities of the different compounds. It seems difficult to correlate any variation in steric hindrance with the activity. Campbell[13] who has tested the corresponding ethane derivatives found them active in the range of 5 to 10 mg as free phenol in the Allen–Doisy test with six subcutaneous injections on castrated rats.

Symmetrical twist of the two α rings

In order to explain the similarity in U.V. absorption between styrene and *unsym*-diphenylethylene, and between *trans*-stilbene and triphenylethylene, Jones[2] assumes that in both cases only one ring is twisted out of the double-bond plane. It will be shown below that such a theory meets several difficulties due to certain experimental facts published later and because cpd III seems to prove the necessity for both rings to support a part of the strain. In spite of these objections, Jones' theory has been until now the most valuable basis for investigations concerning the relationship between U.V. absorption, planarity and estrogenic activity.

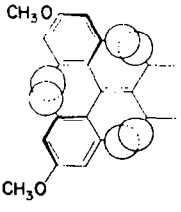
The problem of unsymmetrical twists has been discussed by Ingraham[14] who has compared the estimated energy of a molecule in the case where some rings remain in the plane of the double bond, and in the case where the strain is taken up in as many ways as possible. This last possibility has been found to have more resonance energy and hence predicted to be more stable.

Coates and Sutton[15] have studied the angle formed by the bonds between the phenyl and the α carbon atom in *unsym*-diphenylethylene by electric dipole moment measurements. They found a value greater than the value obtained by calculations but in good accord with bond distance measurements. They concluded both rings must be twisted about 30°.

Interpreting some crystallographic data, Jeffrey *et al.*[3] believe that in *trans–trans*-dienestrol and *trans*-diethylstilbestrol the two rings rotate to the same extent, and remain parallel owing to the existence of centro-symmetry in these molecules.

Braude[8] is against ascribing the E' band to a styrene type of absorption, because in substituted *trans*-stilbene the band at 240 mμ is little affected by substituents, whereas the band at 247 mμ in styrene is displaced to 260 mμ in 4-hydroxystyrene and anethole.

In the cpd III and the ααDE molecule the non-planarity is due not only to the adjacent *ortho* hydrogen atoms of the rings but also to the other *ortho* hydrogen



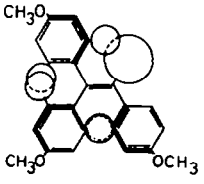
III

[13] N. R. Campbell, *Proc. Roy. Soc.* B **129**, 528 (1940).
[14] L. L. Ingraham, *Steric Effects in Organic Chemistry* p. 490. John Wiley, New York (1956).
[15] G. E. Coates and L. E. Sutton, *J. Chem. Soc.* 567 (1942).

atoms which are hindered by hydrogen atoms from the ethyl group. In such a structure it is not possible for only one of the rings to leave the plane of the double bond; both phenyl rings must contribute to the necessary rotation, and it is reasonable to expect an equal contribution from each of them. Therefore in this β–β di-substituted *unsym*-diphenylethylene it is improbable that a styrene structure plays a part in the *E'* band. This situation may be different in triphenylethylene where one of the rings may be expected to deviate less from the double-bond plane than the others.

U.V. absorption and estrogenic activity in triphenylethylenes

Among the *para*-hydroxylated triphenylethylenes several interesting estrogenic substances have been reported.[16] Trianisylchlorethylene (X) is used clinically under the name chlorotrianisene (N.N.D.). In the study of triphenylethylene, Jones[2] measurements did not go down to the band at 230 mμ. Following his theory one would have attributed this band to a "styrene-like" structure due to its position. Braude would have agreed since the band changes in position and intensity in the methoxy derivatives.



In trianisylethylene (XI) this band appears at 256 mμ with an intensity: log $\varepsilon = 4{\cdot}21$. It is intermediate in position and intensity between the planar *trans*-anethole:[17]

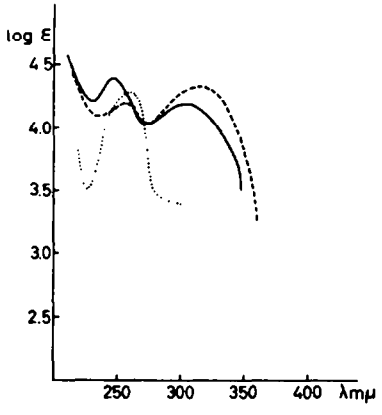


FIG. 2.—trianisylchlorethylene (ethanol); – – – trianisylethylene (ethanol); . . . . anethole (ethanol).

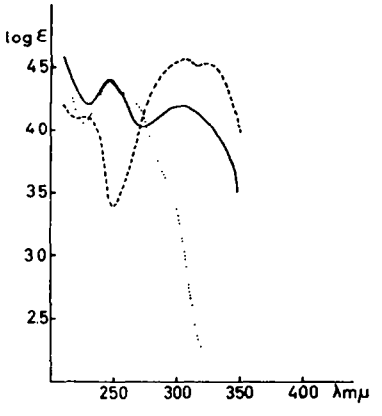


FIG. 3.—trianisylchlorethylene (ethanol); . . . . *unsym*-dianisylethylene (ethanol); – – – di-*p*-methoxy-*trans*-stilbene (ethanol).

259 mμ (log $\varepsilon = 4{\cdot}34$) and the non-planar *cis*-anethole:[17] 253 mμ (log $\varepsilon = 4{\cdot}26$) and so can be attributed to a slightly non-planar anethole moiety (Fig. 2). It seems logical to assign this band to the system of the ethylenic double bond in conjugation with the

[16] R. S. Shelton, M. G. Van Campen, D. F. Meisner, E. R. Permerter, E. R. Andrews, R. E. Allen and K. K. Wyckoff, *J. Amer. Chem. Soc.* **75**, 5491 (1953).
[17] Y. R. Naves, P. Ardizio and C. Favre, *Bull. Soc. Chim. Fr.* 566 (1958).

ring in the *cis* position to the hydrogen atom. (Chart I, structure *A*.) Consideration of the Stuart-molecular models also supports this view.

The other band at 315 mμ (log $\varepsilon = 4{\cdot}34$) can be attributed to a "stilbene like" structure (Chart I, structures *B* and *C*.) However, the intensity is smaller than *trans*-di-*p*-methoxy-stilbene (log $\varepsilon = 4{\cdot}56$). The difference might be caused by the lack of planarity of XI. The presence of the *cis*-stilbene structure must be also considered for this band; *cis*-diethyl stilbestrol diacetate[8] shows three bands respectively at 223 mμ (log $\varepsilon = 4{\cdot}16$) 256 mμ (log $\varepsilon = 4{\cdot}00$) and 278 mμ (log $= 3{\cdot}84$). However, as it will be seen below, the contribution of this structure is probably not important.

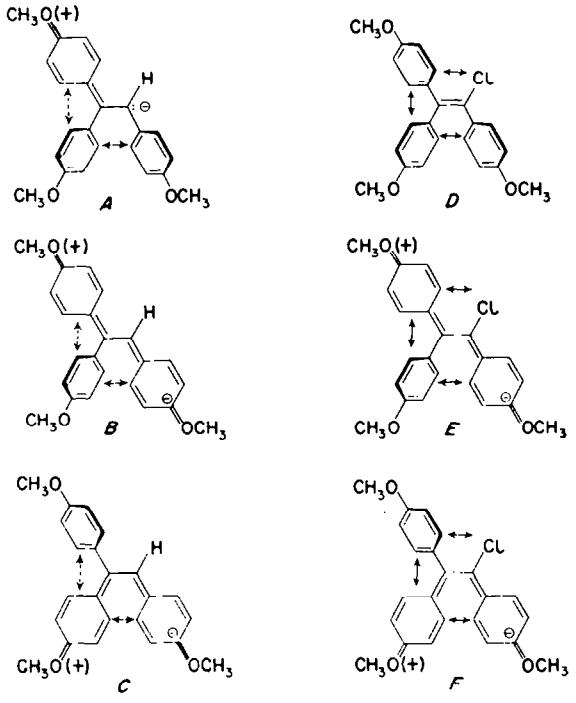


CHART I. Some of the possible resonance structures form 1,1,2-trianisylethylene (*A*, *B*, *C*) and 1,1,2-trianisyl-3-chlorethylene (*D*, *E*, *F*). The symmetrical structures are not drawn. Straight arrow shown represents a strong hindrance; broken arrow represents a less strong hindrance.

The introduction of a chlorine atom at the β-carbon position which increases the estrogenic activity 10 times,[5] provoked a displacement of the anethole-like 256 mμ band to 245 mμ with an increase in intensity to log $\varepsilon = 4{\cdot}40$, which is greater than that of $\alpha\alpha$DE dimethyl ether and *trans*-diethylstilbestrol-dimethyl-ether. The long wavelength band is also shifted to shorter wavelengths but with a decrease in intensity. Thus the band characteristic of conjugation of the same type of structure has disappeared. Hence the structure now seems to be fully non-planar and if the different structures in Chart I: *D*, *E*, *F* are considered, it becomes clear that the new band at 245 mμ must be attributed to an *E'* type band. (Fig. 3.)

The question of the assignment of the long wavelength band remains. As in the

non-chlorinated compounds, the *trans* and *cis* structure must be considered. It is possible to find a partial answer to this problem by considering the fact that the *cis*-stilbene spatial structure (*F*) is not much affected by the introduction of the chlorine atom. Therefore its contribution to the band should remain unchanged and so should the band. Since a hypsochromic shift and a decrease in intensity are observed, the cause is more likely the non-planar *trans*-stilbene structure, which is affected by the chlorine (*E*). Further measurement with *cis* and *trans* substituted triphenylethylenes are necessary to decide the real importance of the contribution of the *cis* structure to the long wavelength band.

As a result of this discussion the short wavelength *E'* band must be attributed to a large extent to the *unsym*-diphenylethylene structure, concurrent with a possible contribution of the non-planar *trans* and *cis* stilbene structure.

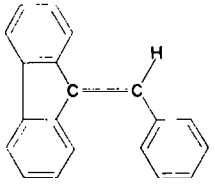
Transformation of the "styrene like" band in trianisyl-ethylene (XI) into a *E'* band in (X) is confirmed by the work of Apelgot *et al.*[18] who have reported the U.V. spectra of triphenylethylene and its β-bromo derivative. Both compounds have their short wavelength maximum at practically the same position (ca. 230 mμ). in spite of differences in planarity. This, however, is due to the lack of methoxyl-groups. In fact the band has a different origin in the two compounds. In the first one it is due to a normal π–π conjugation while in the other it is an *E'* type band.

In the present study the β-substituent of triphenylethylene has been limited to halogen atoms. A verification of these findings should be made by a U.V. investigation of β-alkyltriphenyl-ethylenes[5] and triphenylacrylonitriles.[19] In any case the steric importance attributed to the halogen atom does not mean that its role, as regards the spectrum or the estrogenicity, is limited to this function.

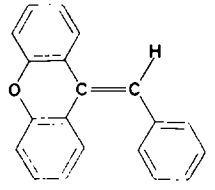
Importance of the two α rings

The estrogenic activity in the two series discussed above seems to depend on a non-planar structure, as with *trans*-diethyl-stilbestrol.

The results of Jacques *et al.* who have found compounds (XII and XIII) in which the α rings are incapable of rotation and which are without estrogenic activity at 100 mg doses in rats, tally well with these conclusions.



XII



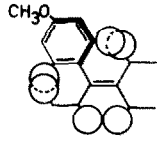
XIII

In order to induce strong estrogenic activity, it is not sufficient that the rings in α and β are both non-planar with respect to the central double bond: *cis*-diethylstilbestrol di-propionate has only 1/600 of the activity of its *trans* isomer,[21] and it has

[18] S. Apelgot, A. Cheutin, S. Mars and M. R. Berger, *Bull. Soc. Chim. Fr.* 533 (1952).
[19] Ng. Ph. Buu-Hoï, L. Corre, A. Lacassagne and S. Lecocq, *Bull. Soc. Chim. Biol.* **23**, 1087 (1947).
[20] J. Jacques, R. Courrier and G. Poumeau-Delille, *Bull. Soc. Chim. Biol.* **27**, 373 (1945).
[21] F. Wessely. A. Bauer, Ch. Chwala, I. Plaichinger and R. Schönbeck, *Monatsh.* **79**, 596 (1948).

been shown that if *cis*-anethole is more active than its planar *trans*-isomer the difference is small.[12]

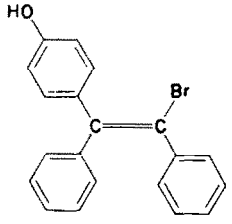
In order to establish whether the second ring is active not merely in inducing non-planar structure but also for more specific reasons compound XIV was prepared. It has the essential features of ααDE (non-planar structure and the same β-substituent). The absorption spectra shows the E_1 band situated at 230 mμ, but the intensity is less than half of that of the ααDE dimethylether, ($\log \varepsilon = 3{\cdot}98$). The shoulder usually found at 270–280 mμ is here transformed into a weaker band at 276 mμ ($\log \varepsilon = 3{\cdot}61$).



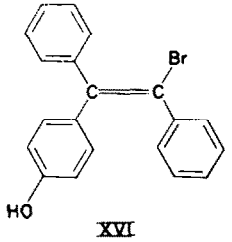
Substance XIV is about 50 times less active in rats than ααDE dimethyl-ether (cpd. III).

These findings also support the importance of the *unsym*-diphenylethylenes structure for estrogenic activity and the fact that this group contributes to the triphenylethylene activity now finds support in the following experimental results previously difficult to explain.

Emmens[22] has prepared the *cis* and *trans* isomers XV and XVI, which produce 50 per cent response in mice, one at 2·7 γ and the other at 3·0 γ. It is well established



XV



XVI

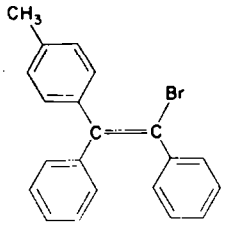
that the presence of an hydroxyl group situated in *para* position on a ring increases considerably the estrogenic activity.* It has also previously been mentioned that a non planar *cis* structure is much less active than a corresponding non planar *trans*-structure. In the case here considered, if the *trans*-stilbene moieties were really determinant for biological activity, the presence of a hydroxyl group on one of the "*trans*" rings in cpd XV would enhance its potency over that of cpd XVI which contains the corresponding *cis*-structure. The lack of a difference in activity between the two compounds indicates the importance of the *unsym*-diphenylethylene part of the molecule.

* J. Jacques (*Bull. Soc. Chim.* 411 (1949)) reports the following figures:

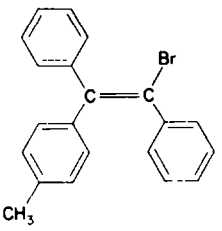
diethylstilbene	inactive 1000 γ
p-hydroxy-diethyl-stilbene	active at 1·2 γ
di-*p*-hydroxy-diethyl-stilbene (stilbestrol)	active at 0·3 γ

[22] C. W. Emmens, *J. Endocriniol.* 3, 120 (1942).

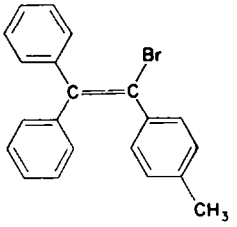
Dvolaitzky and Jacques[23] have recently separated the *cis* and *trans* isomers XVII and XVIII, in which a *para*-methyl group on one of the α rings prevents hydroxylation at that position. In this case too the *cis* and *trans* show nearly the same activity.



XVII



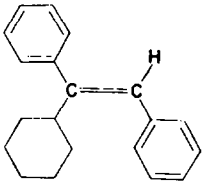
XVIII



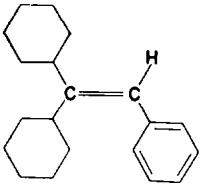
XIX

In spite of the low activity which, as the authors remarked, does not permit any definite conclusions, it is interesting to note that compound XIX, which allows hydroxylation on both α rings, is the most active of the three isomers.

Robson *et al.*[24] have prepared different cyclohexyl analogues of triphenylethylene. Slight activity was found only when two phenyl rings were present in the compound and attached to the same carbon atom.

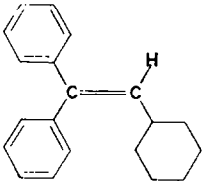


Inactive 20 mg



Inactive 20 mg

The presence of a β-halogen increased the activity of his substance in the same way as the activity of triphenylethylene is increased by the introduction of a halogen.



Active 20 mg

CONCLUSIONS

This study allows us, to include *unsym*-dianisylethylenes and trianisylchlorethylene in a theory based on the "non-planar structure" of the molecule, and in so doing, to make this theory applicable for the most important estrogens in the stilbene series.

[23] M. Dvolaitzky and J. Jacques, *Bull. Soc. Chim. Biol.* **40**, 939 (1958).
[24] J. M. Robson, J. S. H. Davis and W. Tebrich, *Brit. J. Pharmacol.* **5**, 376 (1950).

This study shows also, however, the difficulty in correlating small variations in the planar structure with any appreciable change in estrogenic activity, and a further survey of the literature shows several cases which cannot be explained strictly by a non-planar theory.

In spite of the advances brought by such a theory more refinements have to be introduced in order fully to explain the activity-structure relationships.

In this line an interesting hypothesis has been made by Ōki and Urushibara.[4] They correlate "thickness" and "width" of the molecule, with estrogenic activity. Such a correlation, however, does not seem sufficient to interpret the properties of the compounds under study here, while the thickness of these molecules, probably are almost identical, their width must be quite different, and so also the over-all dimensions of the molecule.

The common factor which seems to be related in a consistent way to the biological properties of our compounds—otherwise rather different from a geometrical point of view—is more probably connected with the presence of the *E'* band. Therefore the electron distribution has to be considered and so the possibilities of conjugation in addition to spatial factors must also be considered in an attempt to correlate structure with estrogenic activity. Whether or not these estrogenically active substances are fixed on the biological receptor merely because the molecule fits the surface spatially, or whether they adhere due to electronically excited groups on the molecule, or at the end of their conjugated system, is not now known.

Resonance theory has been previously applied to explain the activity of several biologically active substances.[25,26]

Carcinogenic substances belonging to the azo-dyes,[26] stilbeneamine,[27] or amino-biphenyl[28] have been discussed in terms of necessary planar structure and consequently maximum conjugation. Those requirements are in contrast to those which the present author considers important for estrogenic activity.

In the stilbene series for example estrogenic activity requires a non-planar structure which reduces conjugation and preferably two hydroxyl groups; carcinogenic activity has more specific requirements: a fully planar conjugated system and only one amino, or amino substituted, group at the end of the conjugated system. This preliminary comparison between estrogenic and carcinogenic active substances based on variation in conjugation is admittedly speculative but may suggest experimental as well as theoretical research.

EXPERIMENTAL

U.V. Absorption. Recordings were made with Beckman Model DU quartz spectrophotometer, using ethanol solution (concentration 10–20 γ per ml).

Nearly all the substances have been described previously; trianisylethylene and its β-chloro derivative were kindly furnished by Wm. S. Merrell Company N.Y.

1-*p*-Methoxyphenyl-1,2,2-tri-ethyl-ethylene (XIV).

10 g (0·05 mole) *p*-methoxy-2-ethyl-butyrophenone in 30 ml anhydrous ether was slowly added to a Grignard reagent prepared with 6 g ethyl bromide and 2 of magnesium turnings in 50 ml anhydrous

[25] P. Meunier, *La Mésomerie* p. 54. Paris (1947).
[26] A. Pullman and B. Pullman, *Cancérisation par les Substarces Chimiques et Structure Moléculaire.* Masson, Paris (1955).
[27] A. Haddow, R. J. C. Harris, G. A. R. Kon and E. M. F. Roe, *Phil. Trans. Roy. Soc.* **241**, 147 (1948).
[28] R. B. Sandin, R. Melby, A. S. Hay, R. N. Jones, E. C. Miller and J. A. Miller, *J. Amer. Chem. Soc.* **74**, 5073 (1952).

ether. After the classical treatment, distillation at 0·5 mm Hg and 95–105° yielded 9 g of a colourless oil (83%). Redistillation at 0·5 mm Hg and 102 gave a colourless, pleasant smelling oil. $n_D^{25} = 1{\cdot}5135$ (Found: C, 82·71: H, 10·27. Calc. for $C_{15}H_{22}O$ (218.33): C, 82·51; H, 10·16%).

Estrogenic activities were determined by the vaginal smear test in ovariectomized mice or rats, the test substances being injected subcutaneously in 0,1 ml olive oil on two succeeding days. The doses shown in Table I, are total doses.

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