Estrogenic Activity of DDT Analogs and Polychlorinated Biphenyls

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Because of the geometric similarity of DDT to the synthetic estrogen, stilbestrol, DDT and 52 related compounds were tested in a sensitive estrogen assay in rats. Estrogenic activity was evaluated using the 18-hr glycogen response of the immature rat uterus. Diphenylmethane, diphenylethane, and triphenylmethane compounds were active when a *p*- or *p'*-position was unoccupied or occupied by an hydroxy or methoxy group. Halide or alkyl groups in the *p,p'*-positions rendered the compounds inactive. Polychlorinated biphenyls and polychlorinated triphenyls, compounds which are environmental pollutants of industrial origin, were estrogenically active. Phenolphthalol and phenolphthalein, compounds which are used as laxatives in drug preparations, were also estrogenically active since they contain the appropriate *p,p'*-dihydroxy structures. Stereo models indicated that *p,p'*-dihydroxy compounds of the active nuclei would have internuclear distances of the hydroxyl groups which would approximate those of the natural steroidal estrogens and the synthetic stilbene estrogens.

In 1945 Solmssen published an excellent and comprehensive 117-page review of the synthetic estrogens and the relation between their structure and activity. In the 25 years since Solmssen's review, DDT, a chlorinated hydrocarbon with a geometric similarity to the synthetic estrogens, has been widely used throughout the world for pest control. The recent demonstration by Welch *et al.* (1969) of the estrogenic activity of *o,p'*-DDT and our own investigations (Bitman *et al.*, 1968) have prompted us to investigate a series of DDT analogs, homologs, and structurally related compounds in an attempt to determine relationships of structure to estrogenic activity.

METHODS

We used the sensitive 18-hr glycogen response of the rat uterus as a measure of estrogenic activity (Bitman *et al.*, 1965). The potency of active compounds is reported in terms of the minimal subcutaneous dose which will increase glycogen to a level significantly different from control. The 18-hr glycogenic response is illustrated in Figure 1, in which the dose-response curve for *o,p'*-DDT is represented. The steeper response line for glycogen, as compared to uterine weight, is readily apparent.

Test substances were dissolved in olive oil or an aqueous ethanol solution and injected subcutaneously at a screening dose rate of 8 mg per rat. Immature female Wistar rats (21–23 days old; 36–48 g) were killed 18 hr after the injection; uteri were quickly excised, weighed, and analyzed for glycogen by the anthrone procedure (Seifter *et al.*, 1950). Substances showing activity were tested further at dosage levels to 0.05 mg. Statistical comparisons were made using Student's t test with correction for unequal group size.

ABBREVIATIONS

Table I: *p,p'*-DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; Tetrachloro-DDT, 1,1,1,2-tetrachloro-2,2-bis(*p*-chlorophenyl)ethane; *p,p'*-DFDT, 1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)ethane; *p,p'*-Perthane, 1,1,1-trichloro-2,2-bis(*p*-ethylphenyl)ethane; *p,p'*-Kelthane, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *p,p'*-DDTF, 1,1,1-trifluoro-2,2-bis(*p*-chlorophenyl)ethane; *p,p'*-DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; *p,p'*-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; *p,p'*-DDMU, 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene; and *p,p'*-DDA, 2,2-bis(*p*-chlorophenyl)acetic acid.

Table II: *o,p'*-DDT, 1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane; *o,p'*-DDE, 1,1-dichloro-2-(*p*-chlorophenyl),2-(*o*-chlorophenyl)ethylene; *o,p'*-DDMU, 1-chloro-2-(*p*-chlorophenyl), 2-(*o*-chlorophenyl)ethylene; *o,p'*-DDD, 1,1-dichloro-2-(*p*-chlorophenyl),2-(*o*-chlorophenyl)ethane; *m,p'*-DDD, 1,1-dichloro-2-(*p*-chlorophenyl),2-(*m*-chlorophenyl)ethane and *p,p'*-Methoxychlor, 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane.

Table III: Compound 20, 1,1-Diphenylmethane; 21, 1,1-Dichloro-1,1-diphenylmethane; 22, 1,1-bis(*p*-bromophenyl)methane; 23, 1-(phenyl)-1-(*p*-hydroxyphenyl)methane; 24, 1,1-bis(*p*-hydroxyphenyl)methane; 25, 1-(*p*-hydroxyphenyl)-1-(*p*-methoxyphenyl)methane; 26, 1-(phenyl)-1-(*p*-methoxy-*o*-hydroxyphenyl)methane; 27, 1,1-bis(*o*-hydroxyphenyl)methane; 28, 1,1-bis(*o*-hydroxy-*m*-chlorophenyl)methane; 29, 1-(phenyl)-1-(*p*-chlorophenyl)-1-methanol; 30, 1,1-bis(*p*-chlorophenyl)-1-methanol; 31, 2,2'-dihydroxybenzophenone; 32, 2,4-dihydroxybenzophenone; 33, 4,4'-dihydroxybenzophenone; and 34, 2,2'-dihydroxy-4,4'-dimethoxybenzophenone.

Sources of the compounds used in this study were: Nos. 4, 5, 16—Rohm and Haas, Philadelphia; 9, 13—Dr. G. F. Fries, U.S. Dept. Agr., Beltsville; 17—E. K. Du Pont de Nemours & Co., Inc., Wilmington; 18—Sigma Chemical Co., St. Louis; 24, 27, 35, 42—K & K Laboratories, Inc., Plainview, N.Y.; 38—Eastman Kodak Co., Rochester; 43–53—Monsanto Co., St. Louis. All other compounds were purchased from the Aldrich Chemical Co., Inc., Milwaukee. Purity, as given by the manufacturers, was better than 99%.

RESULTS AND DISCUSSION

The natural estrogens are steroids which contain a phenolic ring A and an oxygen function at the C_{17} position, while the synthetic estrogens, which are stilbene derivatives, contain two phenolic rings (Figure 2). It is apparent that active estrogenicity is dependent upon the presence of at least one phenolic hydroxy ring structure. In most estrogen tests these compounds are active in the microgram or submicrogram range.

In contrast to this, the chlorinated hydrocarbon pesticides related to DDT are only active as estrogens in milligram amounts, a 1000-fold difference. The DDT analogs are not phenolic, but they may give rise to aromatic phenolic substitution during metabolic conversions in the animal. The

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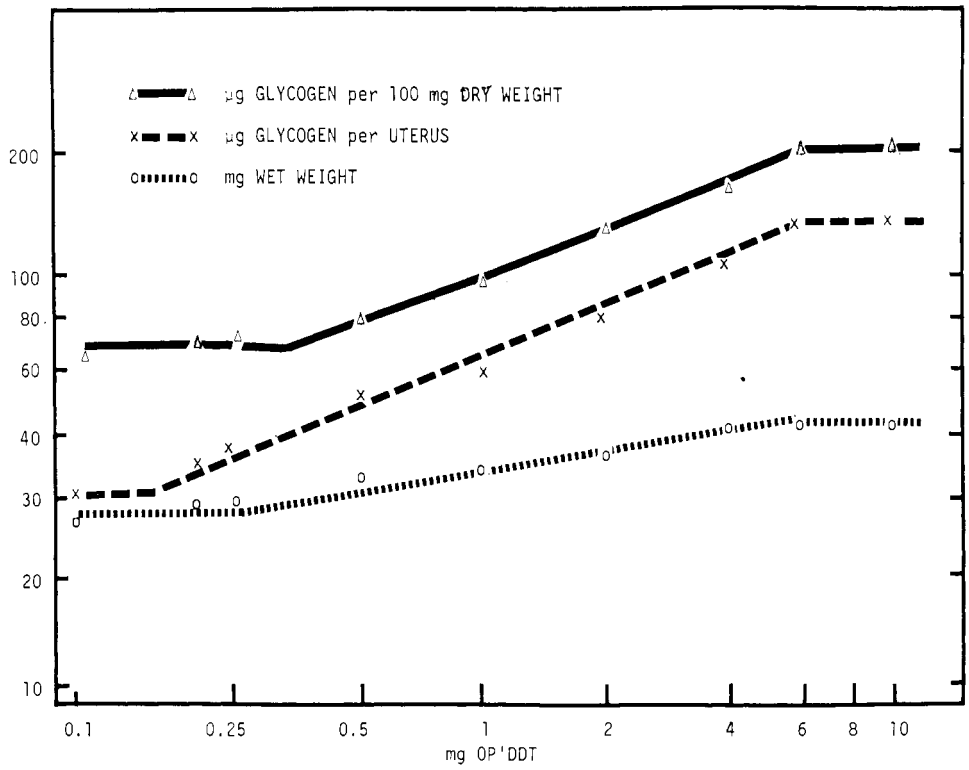


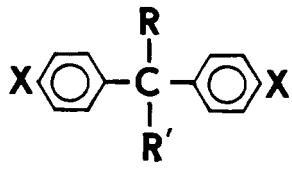
Figure 1. Dose-response relationship: uterine weight, glycogen, and *o,p'*-DDT

DDT analogs are compounds of the diphenylethane type (Figure 2). Other analogs tested were compounds of the diphenylmethane or triphenylmethane series. We have also examined polychlorinated biphenyls and polychlorinated triphenyls, compounds which have become increasingly implicated as environmental pollutants of industrial origin. We have not included in this study any steroids, synthetic estrogens, or anti-estrogens of the stilbene structure, and have excluded almost all compounds of the coumarin, isoflavone, anthracene, and phenanthrene type.

DIPHENYLETHANE COMPOUNDS

***p,p'*-Positions Occupied by Halide or Alkyl.** The compounds evaluated in Table I are diphenylethane derivatives in which the *p,p'*-positions are occupied by halide or alkyl groups. Almost all were devoid of estrogenic activity; *p,p'*-DDT (cmpd 1) and tetrachloro-DDT (cmpd 2) exhibited a slight glycogenic response. It appears that halide or alkyl substitutions in the *p,p'*-positions were stable, and during metabolism in the animal body, little if any *p,p'*-phenolic hydroxy compounds are produced.

Table I. Diphenylethane Compounds with *p,p'*-Positions Occupied by Halide or Alkyl Groups



No.	X	Groups R	R'	Name	Activity M.E.D.[a] mg
1	Cl	H	$-CCl_3$	*p,p'*-DDT	4
2	Cl	Cl	$-CCl_3$	Tetrachloro-DDT	4
3	F	H	$-CCl_3$	*p,p'*-DFDT	I[b]
4	CH_3CH_2	H	$-CCl_3$	*p,p'*-Perthane	I[b]
5	Cl	OH	$-CCl_3$	*p,p'*-Kelthane	I[b]
6	Cl	H	$-CF_3$	*p,p'*-DDTF	I[b]
7	Cl	H	$-CHCl_2$	*p,p'*-DDD	I[b]
8	Cl	...	$=CCl_2$	*p,p'*-DDE	I[b]
9	Cl	...	=CHCl	*p,p'*-DDMU	I[b]
10	Cl	H	—COOH	*p,p'*-DDA	I[b]

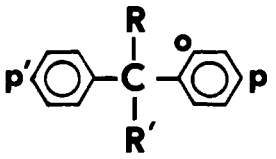
[a] M.E.D. = minimum effective dose. [b] I = inactive.

***p*- or *p,p'* Position Occupied by —H or —OCH_3.** When one of the *para* positions of the aromatic ring is substituted by a hydrogen or methoxy group, the compound exhibits estrogenic activity (Table II). Potency is of a low order of magnitude, being approximately 1000 times less active than compounds of the stilbene series, but is similar in potency to coumarin and isoflavone estrogens (Bickoff *et al.*, 1960). The minimum effective dose (MED) of diethylstilbestrol which elicited a glycogen response was 0.1 µg, as compared to the most active compound of Table II, *o,p'*-DDT, cmpd 11, whose MED was 0.25 mg.

The phenolic character of the natural and synthetic estrogens has demonstrated the dependency of estrogenicity upon the presence of a phenolic structure. The aromatic rings of the active compounds of Table II are open, *i.e.*, they have a *p*- or *p'*-position occupied by —H and may give rise to phenolic substitution during metabolism. There also appears to be a requirement for the ethane chain to be inert, *i.e*, either the trichloroethane ($-CH-CCl_3$) or the vinyl halide group ($>C=CCl_2$) must also be present (cmpds 11, 12, 13). Thus, cmpds 14, 15, and 19, containing more reactive 2-carbon chain configurations, are inactive, even though one of the aromatic rings could be hydroxylated to the phenolic structure. We have concluded that rapid *in vivo* metabolism of these compounds is responsible for their lack of activity.

In the stilbestrol series (Solmssen, 1945) and in the coumarin series (Bickoff *et al.*, 1960) of estrogens, *p,p'*-dimethoxy compounds are less active than comparable *p,p'*-dihydroxy compounds. In the chlorinated diphenylethane series (Table II), the *p,p'*-methoxy compound, methoxychlor (cmpd 17) was

Table II. Diphenylethane Compounds with *p*- or *p'*-Position Occupied by —H or —OCH_3



No.	Groups *p*	*p'*	*o*	R	R'	Name	Activity M.E.D.[a] mg
11	H	Cl	Cl	H	—CCl_3	*o,p'*-DDT	0.25
12	H	Cl	Cl	...	=CCl_2	*o,p'*-DDE	4
13	H	Cl	Cl	...	=CHCl	*o,p'*-DDMU	8
14	H	Cl	Cl	H	—$CHCl_2$	*o,p'*-DDD	I[b]
15	H	Cl	*m*-Cl	H	—$CHCl_2$	*m,p'*-DDD	I[b]
16	H	H	H	H	—CCl_3	1,1,1-Trichloro-2,2-bis(phenyl)ethane	1
17	OCH_3	OCH_3	H	H	—CCl_3	*p,p'*-Methoxychlor	4
18	OCH_3	OCH_3	H	H	—CCl_3	Tech. Methoxychlor (*p,p'*- + *o,p'*-)	1
19	H	H	*o'*-Cl *o*-Cl	H	—CHO	2,2-Bis(*o*-chlorophenyl)acetaldehyde	I[b]

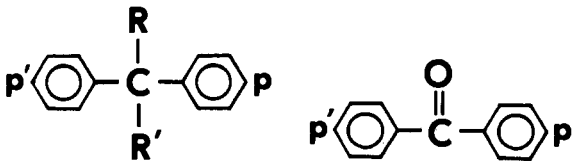
[a] M.E.D. = minimum effective dose. [b] I = inactive.

approximately as active as other compounds which might give rise to phenolic hydroxy substitution on metabolism. Technical methoxychlor (cmpd 18), which may contain an *o,p'*-methoxychlor, was four times more active than pure *p,p'*-methoxychlor.

DIPHENYLMETHANE COMPOUNDS; BENZOPHENONE COMPOUNDS

A series of diphenylmethane compounds was examined to determine structural correlates of estrogenic activity (Table III). Active compounds contained either one or two *p*-hydroxy or *p*-methoxy groups (cmpds 23, 24, 25, 32, 33, 34).

Table III. Diphenylmethane Compounds; Benzophenone Compounds



No.	Groups *p*	*p'*	R	R'	Activity M.E.D.[a] mg
	Diphenylmethane derivatives				
20	H	H	H	H	I[b]
21	H	H	Cl	Cl	I[b]
22	Br	Br	H	H	I[b]
23	H	OH	H	H	2
24	OH	OH	H	H	1
25	OCH_3	OH	H	H	4
26	OCH_3	H	H	H	I[b]
27[c]	H	H	H	H	I[b]
28[d]	H	H	H	H	I[b]
29	H	Cl	H	OH	I[b]
30	Cl	Cl	H	OH	I[b]
31	H	H	...	...	I[b]
	Benzophenone derivatives				
32	H	OH	...	...	2
33	OH	OH	...	...	2
34	OCH_3	OCH_3	...	...	4

[a] M.E.D. = minimum effective dose. [b] I = inactive. [c] bis(*o*-hydroxyphenyl)methane. [d] bis(*o*-hydroxy, *m*-chlorophenyl)methane.

The most active compound was *p,p'*-dihydroxydiphenylmethane which elicited a glycogenic response at the 1 mg dose level. Solmssen (1945) reported activity for this compound at the 100 mg level, but the differences in bioassay procedures could explain part of this difference in result.

In the diphenylethane series, compounds with a *p*-hydrogen and a stable ethane chain were metabolized to active estrogens, probably containing a *p*-hydroxy structure. In contrast, diphenylmethane compounds with a *p*-hydrogen were not active, probably being metabolized rapidly at the methane linkage and excreted from the body.

Benzophenone derivatives, which contain the more stable ketone structure at the methane carbon, were active if a *p*-hydroxy was present (Table III).

DIPHENYL PROPANE COMPOUNDS; TRIPHENYL METHANE COMPOUNDS

Two diphenylpropane compounds were active (cmpds 35 and 36), the *p,p'*-hydroxy compound exhibiting much greater activity than a *p,p'*-dimethoxy compound. Dihydroxy diphenyl propane (cmpd 35) was as active as *o,p'*-DDT. Solmssen (1945) found that this compound was active at a 100 mg dose level.

Since the *p,p'*-dihydroxy structure appeared to be the structure conferring activity, phenolphthalol, a phenyl substituted diphenylmethane compound containing *p,p'*-dihydroxy groups was tested. Phenolphthalol was as potent as any compound of the types studied. Ring closure, as in phenolphthalein, resulted in a 20-fold loss in potency. These compounds are not known to have estrogenic activity and are extensively used as laxatives in a number of drug preparations.

In Table IV two miscellaneous derivatives which bear some relation to closed ring diphenylmethane structures are included: fluorene and 9,10-dimethylanthracene. Both of these compounds were inactive when tested at dose levels up to 8 mg per rat.

BIPHENYL AND TRIPHENYL COMPOUNDS

Two hydroxy biphenyl compounds were active but only at the 4 and 8 mg dose levels (Table V). In a series of polychlorinated biphenyls, the compounds containing up to 48% chlorine were active. As judged from glc chromatograms,

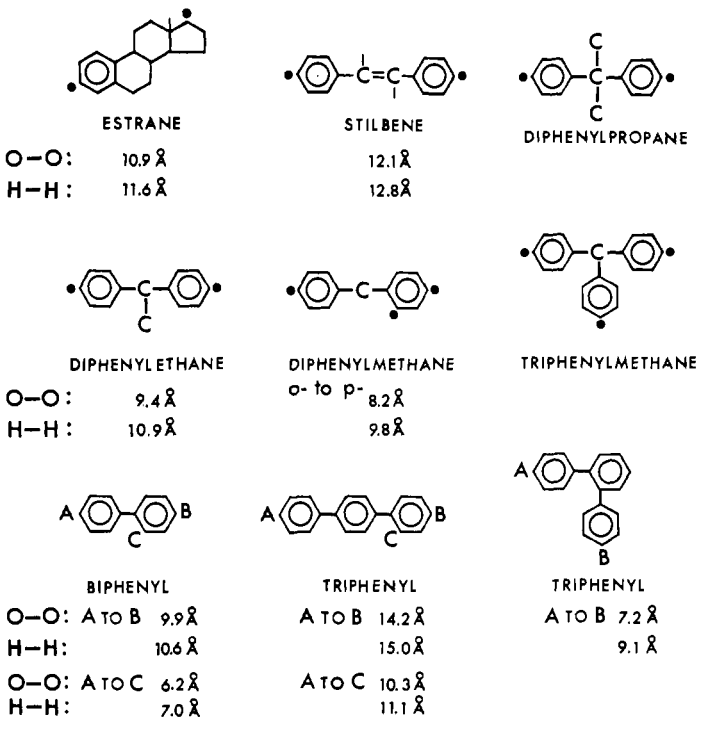


Figure 2. Structural formulae of estrogenic compounds

these products are crude mixtures containing a number of compounds. A polychlorinated triphenyl containing 42% chlorine was found to be more active, at a 1 mg dose level.

CORRELATIONS BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY

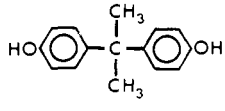
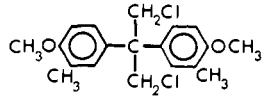
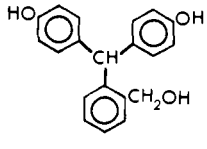
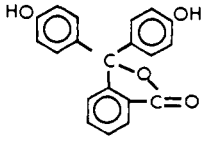
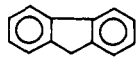
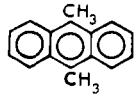
Schueler (1946) and his coworkers (Fisher *et al.*, 1952; Keasling and Schueler, 1950) have theorized that a rather large, rigid, lipoid soluble molecular structure with two active hydrogen-bond forming groups located at an optimum distance of 14.5 Å units from each other would be estrogenic. They further stated that potency is decreased as the distance between groups is decreased or increased.

While DDT possesses a relatively large, rigid, lipoid soluble molecular constitution, it does not present active hydrogen atoms at the hypothesized optimum distance of 14.5 Å, however. The presence of the electronegative chlorine atoms in the *p,p'*-orientations would prohibit the existence of active hydrogen. If these *p,p'* chlorine atoms were metabolized to groups possessing active hydrogen, the possibility of estrogen action would exist. The general lack of estrogenic activity of *p,p'*-DDT analogs suggests that such metabolism does not occur readily in the biological situations studied thus far.

Conversely, the activity of *o,p'*-DDT raises interesting theoretical relationships between chemical constitution and estrogenic activity. The *o,p'* chlorine atoms are not at the hypothesized optimum distance. The exact nature of the active estrogen structure arising from *o,p'*-DDT, if it is not *o,p'*-DDT itself, might provide important information relating to the spatial configuration of an active estrogen.

Dreiding Stereomodels were constructed of many of the active structures to determine whether consistent stereochemical factors were present. Internuclear distances were measured and were given in Figure 2 for both O to O atoms of assumed dihydroxy compounds, and for the H to H distance of the hydroxyl groups. Interatomic distances for the Dreiding models of estrane and stilbene were found to be much

Table IV. Diphenylpropane and Triphenylmethane Compounds

No.	Formula	Name	Activity M.E.D.[a] mg
35		2,2-Bis(*p*-hydroxyphenyl)propane	0.25
36		1,3-Dichloro-2,2-bis(*p*-methoxy, *m*-methylphenyl)-propane	4
37		Phenolphthalol	0.2
38		Phenolphthalein	4
39		Fluorene	I[b]
40		9,10-Dimethylanthracene	I[b]

[a] M.E.D. = minimum effective dose. I = inactive.

Table V. Biphenyl and Triphenyl Compounds

No.	Name		Activity M.E.D.[a] mg
41	*o,o'*-Biphenol	2,2'-Dihydroxydiphenyl	4
42	*p,p'*-Biphenol	4,4'-Dihydroxydiphenyl	8
	Polychlorinated Biphenyl (PCB)		
43	PCB Aroclor 1221	21% Chlorine	8
44	PCB Aroclor 1232	32% Cl	8
45	PCB Aroclor 1242	42% Cl	8
46	PCB Aroclor 1248	48% Cl	8
47	PCB Aroclor 1254	54% Cl	I[b]
48	PCB Aroclor 1260	60% Cl	I[b]
49	PCB Aroclor 1262	62% Cl	I[b]
50	PCB Aroclor 1268	68% Cl	I[b]
51	PCB Aroclor 4465	60% PCB, 40% polychlorinated triphenyl (PCT), 65% Cl	I[b]
52	PCT Aroclor 5442	42% Cl	1
53	PCT Aroclor 5460	60% Cl	I[b]

[a] M.E.D. = minimum effective dose. [b] I = inactive.

smaller than the 14.5 Å quoted by Keasling and Schueler (1950). The 10.9 Å we found agree closely with the X-ray crystallographic data of Norton *et al.* (1963, 1964), who found 10.95 Å for 17β-estradiol. This discrepancy in interatomic differences may be related to the improved accuracy of the current atomic models, when compared to those used in 1950.

The diphenylethane, diphenylmethane, diphenylpropane, triphenylmethane, biphenyl, and triphenyl compounds all have interatomic distances of 9.4 to 10.3 Å for the most likely O to O substitutions. The H to H internuclear distances of the hydroxyl groups range from 9.1 to 11.1 Å in these compounds. Both the O—O and H—H internuclear distances, therefore, are only slightly smaller than corresponding bond distances in natural and synthetic estrogens.

The structural observations regarding estrogenic activity in the compounds studied indicated that activity is conferred when a *p*- or *p'*-position is unoccupied (—H), or is substituted by —OH or —OCH_3. Halide, or alkyl groups, occupying the *p,p'*-positions render the compounds estrogenically inactive. A stable ethane chain was found to be necessary for activity, *e.g.*, the trichloroethane or the inert vinyl halide group; if either C of the ethane chain bears an oxygen function (alcohol, aldehyde, or acid), the compound is metabolized and no estrogenic activity is observed. Some polychlorinated biphenyl and triphenyl compounds exhibited estrogenic activity. Measurements of internuclear distances of Dreiding steric models indicated that active sites would be 9–11 Å apart, a range similar to those found in natural and synthetic estrogens. Quantitatively similar estrogenic activity was obtained with a series of diphenylmethane or triphenylmethane derivatives which contained *p*-OH functions. Correlations of structure with activity suggest that the active estrogens derived from *o,p'*-analogs of DDT are *p*-phenolic metabolites.

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