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CHEMICAL CONSTITUTION AND SEX-HORMONE ACTIVITY: SOME ANALOGUES OF TRIPHENYLETHYLENE

BY

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It would be valuable, in certain conditions, to be able to antagonize the actions of oestrogens in the body; thus, for example, oestrogen antagonists might be the optimum form of treatment in certain types of menorrhagia. We have attempted to produce such substances by making derivatives and analogues of triphenylethylene. It was hoped that these would, by a mechanism of substrate competition, prevent the action of the oestrogen on the effector organ. Triphenylethylene was chosen as the parent oestrogenic substance because it has a fairly simple structure, and derivatives and analogues of it can be fairly readily made. The results of the investigation have, unfortunately, been negative, but the results reveal interesting effects of certain chemical groupings on the activity of this group of oestrogenic substances, and may also prove of help to other investigators who wish to pursue a similar line of work.

METHODS

Determination of oestrogenic activity.—The substances were dissolved in oil and administered to groups of four or five ovariectomized mice by subcutaneous injection. Each mouse received the total dose in four injections given on the morning and evening of two consecutive days. With one substance (No. 65), which was an oil, one test was done by giving 50 mg. of the substance as a single subcutaneous injection. Smears were made on the evening of the next day and on the next two days, and for longer periods if positive results were being obtained.

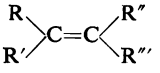
Determination of anti-oestrogenic activity.—The anti-oestrogenic activity of the substances was tested against (i) a derivative of triphenylethylene, viz. D.B.E. (2-bromo-2-phenyl-1 : 1-di-(*p*-ethoxyphenyl) ethylene) and (ii) the natural oestrogen, oestradiol.

Each test was performed on five groups of five mice. Group 1 received 0.1 μg. of oestradiol in oil in four subcutaneous injections (on the morning and evening of two consecutive days); group 2 received 10 μg. of D.B.E. in 0.1 c.c. oil by stomach tube as a single dose on the morning of the first day; group 3 received 0.1 μg. of oestradiol in four doses together with the substance to be tested, dissolved in oil and also given in four doses; group 4 received 10 μg. D.B.E. by stomach tube and the substance to be tested in the same way as in group 3; group 5 received the substance to be tested. When two or more substances were tested at the same time for anti-oestrogenic activity, only one series of controls was used. Smears were made from all animals on the evening of the day following the last injection and on the two following days.

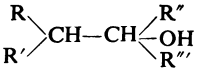
CHEMICAL SECTION

(Analyses are by Drs. Weiler and Strauss, Oxford ; melting points are uncorrected.)

The compounds tested were all ethylene derivatives of the general type (I), where one substituent was usually hydrogen, except in Nos. 5, 65, and 200 ; the other substituents were phenyl-, *p*-alkoxyphenyl-, *cyclo*hexyl-, thienyl-, *p*-dimethylaminophenyl-, and ethylthio- groups.



(I)



(II)

They were prepared by dehydration of the carbinols (II), obtained by the action of a Grignard compound on the appropriate ketone or ester. The intermediate carbinols were not always isolated in a pure state ; they were dehydrated by heating with anhydrous formic acid or, more usually, by distillation *in vacuo* with a few drops of 20% sulphuric acid. The solid ethylenes were crystallized from methanol or ethanol.

Table I gives a list of the compounds tested with their serial numbers, melting or boiling points, and analyses. Notes are appended on their preparation.

TABLE I

No.	Ethylene derivatives*	B.P. °C.	M.p.°C.	Formula	Found % C.	Found % H.	Required % C.	Required % H.
1	1-*cyclo*Hexyl-1 : 2-diphenyl-	137–138/0.3 mm.	—	$C_{20}H_{22}$	91.3	8.6	91.6	8.4
3	1 : 1-*di*Cyclo*hexyl*-2-*phenyl*-	150–151/4 mm.	39	$C_{20}H_{2}$	89.0	10.5	89.5	10.5
4	2-cyclo*Hexyl*-1 : 1-*diphenyl*-	134/0.25 mm.	50	$C_{20}H_{22}$	91.4	8.7	91.6	8.4
5	2-*Bromo*-2-cyclo*hexyl*-1 : 1-*diphenyl*-	—	112	$C_{20}H_{21}Br$	70.4	6.2	70.4	6.2
38	1 : 2-Diphenyl-1-(2′-thienyl)-	165–170/0.7 mm.	72	$C_{8}H_{14}S$	82.0	5.3	82.4	5.3
40	2-*Phenyl*-1 : 1-*di*-(2′-*thienyl*)-	175–182/2 mm.	75	$C_{1}H S_{.}$	72.1	4.7	71.7	4.5
49	2-*Phenyl*-1-(p-*methoxyphenyl*)-1-(2′-*thienyl*)-	204/0.75 mm.	73.5	C H OS	78.0	5.5	78.1	5.5
50	2-(p-*Ethoxyphenyl*)-1 : 1-*di*-(2′-*thienyl*)-	220–221/1.9 mm.	65	$C_{1}.H_{1}OS_{2}$	68.85	5.0	69.2	5.1
54	1-*Phenyl*-2-(p-*ethoxyphenyl*)-1-(2′-*thienyl*)-	200–210/0.1 mm.	87	$C_{20}H_{1}.OS$	78.2	6.15	78.45	5.9
55	1-(p-*Methoxyphenyl*)-2-(p-*ethoxyphenyl*)-1-(2′-*thienyl*)-	242/2 mm.	105	$C_{21}H_{20}O_{2}S$	74.6 S, 9.6	6.3	75.0 S, 9.5	5.95
65	1 : 2-*Diethyl*-2-*phenyl*-1-(2′-*thienyl*)-	154/10 mm. 136/1 mm.	—	$C_{1}H_{18}S$	79.35 S, 12.8	7.6	79.35 S, 13.2	7.4
69	2-Phenyl-1 : 1-di-(*p*-dimethylaminophenyl)-	—	132	—	—		—	
200	2-*Bromo*-2-*phenyl*-1 : 1-*di*-(p-*ethylthiophenyl*)-	—	74	$C_{2}H_{2}.S_{2}Br$	63.7	5.0	63.3	5.3

* The names of new compounds are printed in italic type.

Notes on the preparations

No. 1. From deoxybenzoin and *cyclo*hexylmagnesium chloride. Unchanged deoxybenzoin removed with Girard reagent T.

No. 3. From di*cyclo*hexyl ketone (Breslow, Walker, Yost, and Hauser, 1945) and benzylmagnesium chloride.

No. 4. From ethyl *cyclo*hexylacetate and phenylmagnesium bromide. Benzophenone and *cyclo*hexylmethylmagnesium bromide (Hiers and Adams, 1926) gave benzhydrol as the main product.

No. 5. By bromination of No. 4 in boiling acetic acid.

No. 38. From 2-phenylacetylthiophen (" Organic Syntheses," Coll. Vol. II, 520) and phenylmagnesium bromide. Buu-Hoi and Hiong-Ki-Wei (1945) give m.p. 68° C. for the product from 2-benzoylthiophen and benzylmagnesium chloride.

No. 40. From 2-phenylacetylthiophen and 2-thienylmagnesium iodide. The intermediate 2-*phenyl*-1 : 1-*di*-(2′-*thienyl*)*ethane*-1-*ol* crystallized from light petroleum (b.p.

60–80° C.) in short prisms, m.p. 78° C. (Found: C, 67.1; H, 5.3. $C_{16}H_{14}OS_2$ requires C, 67.1; H, 4.9%).

No. 49. From 2-phenylacetylthiophen and *p*-methoxyphenylmagnesium bromide, or from 2-p-*methoxybenzoylthiophen* and benzylmagnesium chloride. The intermediate 2-*phenyl*-1-(p-*methoxyphenyl*)-1-(2′-*thienyl*)*ethan*-1-*ol* had m.p. 74° C. (Found: C, 73.3; H, 5.8. $C_{19}H_{18}O_2S$ requires C, 73.6; H, 5.8%). 2-*p*-Methoxybenzoylthiophen, needles from methanol, m.p. 73° C. (Found: C, 65.8; H, 4.7. $C_{12}H_{10}O_2S$ requires C, 66.05; H, 4.6%) was prepared from *p*-methoxybenzoyl chloride and thiophen by the Friedel-Crafts reaction.

No. 50. From ethyl *p*-ethoxyphenyl acetate and 2-thienylmagnesium iodide.

No. 54. From 2-p-*ethoxyphenylacetylthiophen* and phenylmagnesium bromide. The intermediate 1-*phenyl*-2-(p-*ethoxyphenyl*)-1-(2′-*thienyl*)*ethan*-1-*ol* crystallized in needles from light petroleum, m.p. 83° C. (Found: C, 74.2; H, 6.5. $C_{20}H_{20}O_2S$ requires C, 74.1; H, 6.2%). 2-*p*-Ethoxyphenylacetylthiophen, colourless prisms from methanol, m.p. 85° C. (Found: C, 68.25; H, 5.8. $C_{14}H_{14}O_2S$ requires C, 68.3; H, 5.7%) was prepared from *p*-ethoxyphenylacetyl chloride, b.p. 106° C./0.5 mm., and thiophen by the Friedel-Crafts reaction in carbon disulphide using aluminium chloride.

No. 55. From 2-*p*-ethoxyphenylacetylthiophen and *p*-methoxyphenylmagnesium bromide.

No. 65. From 2-*α*-phenyl-*n*-butyrylthiophen and ethylmagnesium bromide (cf. Buu-Hoi and Hiong-Ki-Wei, 1945).

No. 200. By brominating in glacial acetic acid 2-*phenyl*-1:1-*di*-(p-*ethylthiophenyl*)-*ethan*-1-*ol*, needles from aqueous ethanol, m.p. 98° C. (Found: C, 73.0; H, 6.4. $C_{24}H_{26}OS_2$ requires C, 73.1; H, 6.6%), prepared from p:p′-*diethylthiobenzophenone* and benzylmagnesium chloride. *p*:*p*′-Diethylthiobenzophenone, m.p. 118° (Found: C, 67.1; H, 6.2. $C_{17}H_{18}OS_2$ requires C, 67.5; H, 6.0%) was obtained in 15–16% yield from ethylthiophenol and oxalyl chloride after the method of Schönberg (1924).

RESULTS

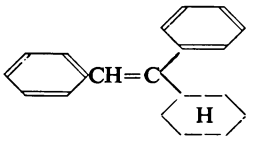
All substances tested for possible anti-oestrogenic activity proved to have no such effect. Three of the substances (Nos. 4, 5, and 65) had some oestrogenic activity. With a dose of 20 mg. the oestrogenic effect of No. 5 decreased to half in 15 days, when measured by the method of Robson (1938); 1 mg. produced cornification in all mice, but the effect was of short duration. Substance 65, in a dose of 20 mg., produced a definite oestrogenic effect, though none of the mice receiving that dose were fully cornified. With 50 mg. all mice were fully cornified and the duration to half action was 21 days. Compound 4 produced full cornification of short duration in two out of five mice. No. 54 was not tested for anti-oestrogenic activity, since its structure is very similar to other substances which were tested and found to have no such effect.

DISCUSSION

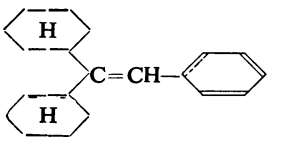
The pharmacological results show quite clearly that none of the substances tested inhibits the action of D.B.E. and oestradiol on the vagina. Exactly the same method has been used previously to demonstrate the inhibition of the action of oestrone and oestradiol by progesterone and testosterone (Robson, 1938), and hence it can at least be said that the method is capable of demonstrating such an inhibitory effect. A different type of inhibition of the action of oestrogen has been demonstrated by Hertz (1948), using a folic acid antagonist. This question requires further investiga-

tion, but it seems likely that the folic acid antagonist does not directly inhibit the action of the oestrogen, but inhibits the taking up of folic acid in some process necessary for the growth of the tissue stimulated by the oestrogen.

The oestrogenic activity of certain of the compounds tested shows points of interest. The first approach to the production of the anti-oestrogen was by the substitution of *cyclo*hexyl for phenyl rings in the triphenylethylene molecule. It was found that such substitution markedly decreased or abolished the activity. Slight activity was found only when two phenyl rings were present in the compound and both were attached to the same carbon atom (No. 4).



No. 1. Inactive (20 mg.)



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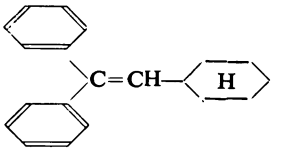
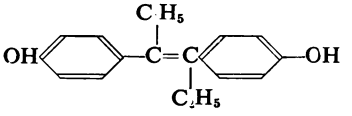


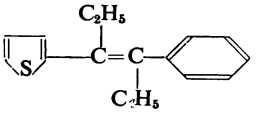
No. 4. Active (20 mg.).

It is also of interest that the presence of a halogen increased the activity of this substance in the same way as the activity of triphenylethylene is increased by the introduction of a halogen.

The preparation of compounds containing the thiophen group was prompted by the publication of the paper of Buu-Hoi and Hiong-Ki-Wei (1945), in which thiophen derivatives of stilbene are described. The introduction of one or two thiophen groups abolishes oestrogenic activity, with one exception. This is No. 65 which has a superficial resemblance to stilboestrol and has a slight oestrogenic activity.



Stilboestrol.



No. 65.

In a comparable investigation on some 1-*cyclo*pentyl- and 1-*cyclo*hexyl-1 : 2-diarylethylenes, Hey and Musgrave (1949) have reported likewise that none of the ethylenes tested showed any significant oestrogenic or anti-oestrogenic activity.

It became of interest to investigate the influence of dimethylamino groups as substituents, and for this purpose 2-phenyl-1 : 1-di-(*p*-dimethylaminophenyl)ethylene (No. 69), prepared as described by Kehlstadt (1944), was tested, but here again no oestrogenic activity was shown.

To determine the effect of replacement of oxygen by sulphur, the sulphur analogue of D.B.E., viz., 2-bromo-2-phenyl-1 : 1-di(*p*-ethylthiophenyl)ethylene (No. 200), was compared directly with D.B.E. for oestrogenic activity and was found to be inactive

at a dose of 100 μg. administered orally, whereas D.B.E. showed marked activity at this dose. It was also found to be inactive as an anti-oestrogen. For this test, our thanks are due to Miss Audrey Hudson.

SUMMARY

The sex-hormone activity of some analogues of triphenylethylene, containing *p*-alkoxyphenyl-, *cyclo*hexyl-, thienyl-, *p*-dimethylamino-, and *p*-ethylthio- groups, has been examined. In all compounds, the replacements markedly decreased or abolished oestrogenic activity and, where tested, this was not accompanied by the emergence of any anti-oestrogenic activity.

We would express our indebtedness to Professor D. H. Hey, who initiated the chemical work of this communication, and to the Medical Research Council for a grant (to J. M. R.) from which the expenses of this investigation have, in part, been defrayed.

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