REVIEW ARTICLE

SYNTHETIC ŒSTROGENS

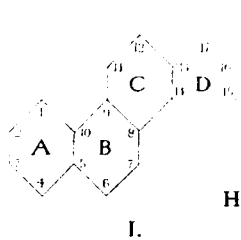
By E. C. Dodds,

M.V.O., M.D., D.Sc., F.R.C.P., F.R.S.

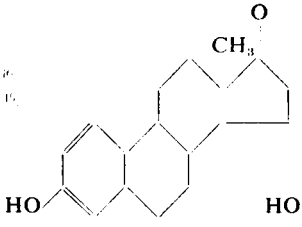
Courtauld Professor of Biochemistry in the University of London

The field of chemotherapy in general has shown the large numbers of compounds which can be used for the same purposes. One only has to contemplate the development of the so-called "sulpha" drugs to provide an example of this. The first compound produced by Domagk[1] was the complex dye-stuff Prontosil. This substance obtained wide acceptance for the treatment of certain infections, but almost completely disappeared after the brilliant observations of Trefouel, Nitti and Bovet[2] that in the rabbit the compound was split at the azo linkage, liberating sulphonamide, and that this simple substance itself was active against certain infections. As we know, this was the start of a whole series of drugs of which there must be by now many hundreds on the market.

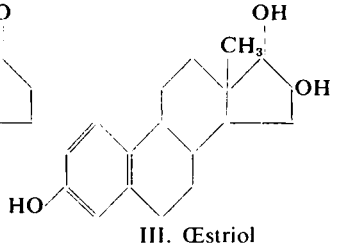
The same story can be told of the anti-syphilitic remedies, and the same is true of the anti-malarials. The physician wishing to treat either of these conditions has a wide range of compounds to choose from, many of them differing quite fundamentally in constitution, despite the fact that they all bring about the same therapeutic results.



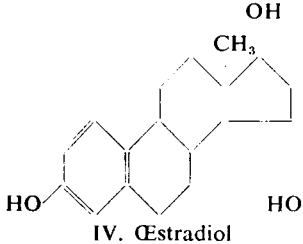
I.



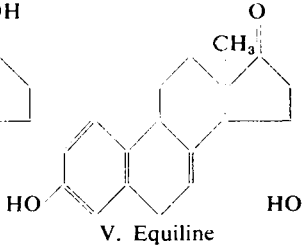
II. Œstrone



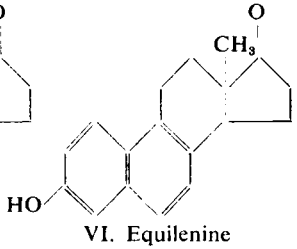
III. Œstriol



IV. Œstradiol



V. Equiline



VI. Equilenine

Up to the discovery of synthetic œstrogens, this phenomenon did not apply to the hormones. There has been only one adrenaline isolated from the suprarenal medulla, only one thyroxine from the thyroid gland and only one insulin from the pancreas.

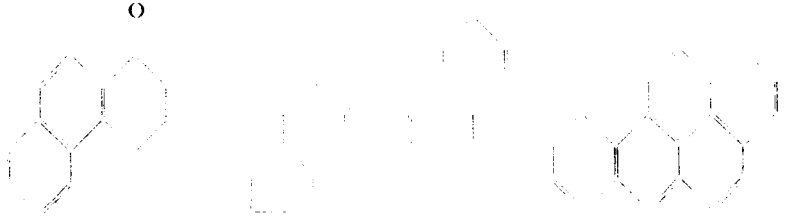
The first indication that there might be a whole series of closely related substances secreted by one endocrine gland came with the isolation of five different œstrogenic substances from the urine of pregnancy, namely, œstrone, œstriol, œstradiol, equiline and equilenine (II to VI). These all have the same qualitative effect, though their quantitative effects are different.

The same story was unfolded concerning the suprarenal cortex. It is now known that a very large number of steroid substances are produced by the cortex of the suprarenal gland, differing in constitution and differing considerably in their metabolic action.

Chemistry

Up to the present time the basic structure of the cyclopentenophenanthrene ring system (I) has been found indispensable for androgenic, progestational and adreno-cortical action. In the case of œstrogens it has been found possible to break away from this structure.

It is only necessary to review very briefly the work leading to the synthesis of stilbœstrol and its allied compounds. Experiments were begun in the Courtauld Institute about 1930 with the object of seeing how far it was possible to change the molecule of œstrogenic substances without destroying the biological activity. As all naturally-occurring œstrogens contain the phenanthrene system as part of the nucleus, a number of phenanthrene derivatives were prepared and tested by the vaginal smear method (Stockard and Papanicolaou[3]) on ovariectomised rats. In 1933, the substance 1-keto-1:2:3:4-tetrahydro-phenanthrene (Cook, Dodds and Hewett[4]) was found to be active in rats at a dose level of 100 mg./rat (VII). At the same time a certain similarity was noticed between the microscopic appearance of the cells of the vagina under the influence of œstrogens and the proliferation caused by the painting of carcinogenic hydrocarbons on the skin. Two of the most potent carcinogenic hydrocarbons, 5:6-cyclopenteno-1:2-benzanthracene (VIII) and 1:2-benzpyrene (IX) were tested and found to have definite,



VII. 1-keto-1:2:3:4-tetrahydro-phenanthrene

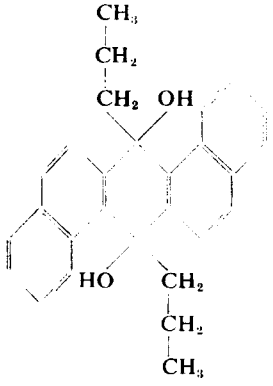
VIII. 5:6-cyclopenteno-1:2-benzanthracene

IX. 1:2-benzpyrene

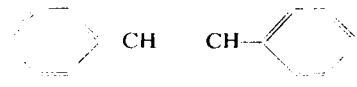
though slight œstrogenic activity (Cook and Dodds[5]). Moreover, it was found that by introducing groups in the 9:10-position of dibenzanthracene this could be converted into quite a powerful œstrogen. A series of 9:10-dihydroxy-9:10-dialkyl-1:2:5:6-dibenzanthracenes was specially

investigated, and the di-*n*-propyl substituent (X) was found to be active in a dose of 25 μg. in the rat (Cook, Dodds, Hewett and Lawson[6]).

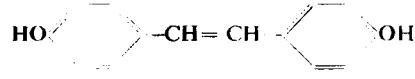
At this point it was decided to see whether the phenanthrene nucleus could be dispensed with and a series of compounds was made, the aim always being to find the simplest possible substance with the highest œstrogenic activity. Considerable activity was shown by certain compounds with only two benzene rings, particularly by stilbene (XI) and 4:4′-dihydroxystilbene (XII) (Dodds and Lawson[7]). An attempt was then made to "drop" one of the rings and the compound anol, *p*-hydroxypropenylbenzene (XIII), was tested. This appeared to be very highly active (Dodds and Lawson[8]), but when other workers attempted to repeat the observation, considerable variation was found in the different batches of anol, some having only very slight activity. The conclusion was that the activity in some batches of anol was due to



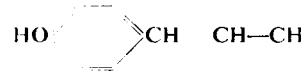
X. 9:10-Dihydroxy-9:10-di-*n*-propyl-1 : 2 : 5 : 6-dibenzanthracene



XI. Stilbene



XII. 4 : 4′-Dihydroxystilbene



XIII. Anol

a contaminant, probably a dimeride of anol. The unsymmetrical dimeride, di-anol (XIV), was tested, but though active, it was not sufficiently so to account for the high activity of some of the batches of anol (Campbell, Dodds and Lawson[9]). The other possibility was the symmetrical dimeride, 4:4′-dihydroxy-α:β-diethyl stilbene, later known as stilbœstrol (XV). This compound was synthesised by a combined team from Sir Robert Robinson's Department at Oxford and from the Courtauld Institute. When tested on rats by the vaginal smear method this was found to be the most powerful œstrogenic substance then known (Dodds, Golberg, Lawson and Robinson[10]). At the same time it was found possible to isolate another compound from the residue remaining from the anol crystallisation, and this compound was later known as hexœstrol (XVI) (Campbell, Dodds and Lawson[11]). A further compound, dienœstrol (XVII), was made a few months later (Dodds, Golberg, Lawson and Robinson[12]).

These compounds have now been used, particularly for the treatment of menopausal symptoms, for nearly ten years, and have been found to

replace the naturally-occurring œstrogens in every way, with the additional advantage that they are active by mouth. They have also been used since the publication by Huggins[13] of his observations on carcinoma of the prostate for the treatment of this condition, and have proved to be of great benefit in a large proportion of cases.

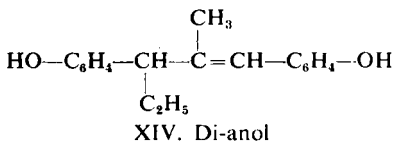
With the establishment of stilbœstrol, hexœstrol and dienœstrol as therapeutic agents, it looked as if the interest in this particular field had more or less come to an end. Recently, however, there have been a number of developments of entirely new synthetic œstrogens, and it is mainly with these that the present account is concerned.

In the first instance, we must abandon the use of the term " synthetic œstrogen," as pointed out recently by Horeau[14]. The synthesis of œstrone has now been effected, and therefore the naturally-occurring hormone could also be included under the heading of synthetic œstrogens. For the stilbœstrol type of substance it is better to employ the term " artificial œstrogens " in the future.

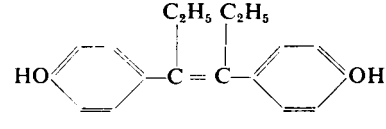
The total synthesis of œstrone was effected by Anner and Miescher[15]. Whilst this is of great theoretical importance, it would appear very unlikely that the synthetic product will ever compete with the production from natural sources. The natural œstrogens are prepared commercially either from the urine of certain pregnant animals, notably the mare, or from cholesterol by a degradation synthesis.

The fact that there were available three artificial œstrogens for use in therapeutics did not hinder the attempts to find others, since it was hoped by this means to find some clue as to the reason for the œstrogenic activity shown by substances with a constitution far different from that of the natural product.

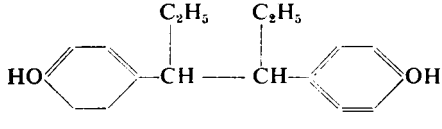
Recently, clinical interest has been shown in a substance produced by Inhoffen and Hohlweg[16] as long ago as 1938. These workers showed



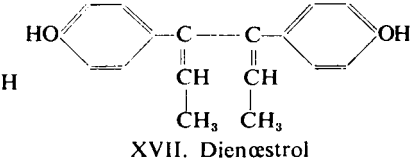
XIV. Di-anol



XV. Stilbœstrol



XVI. Hexœstrol

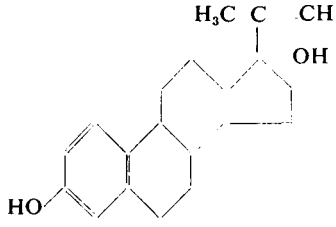


XVII. Dienœstrol

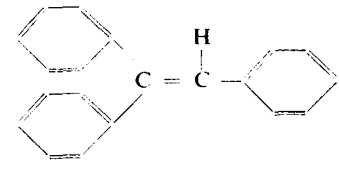
that it was possible to introduce an ethylenic linkage on to the 17-carbon atom in œstradiol. The resulting compound was called ethinyl œstradiol (XVIII). This derivative of the naturally-occurring substance was found to be active by mouth, but it was also stated to suffer from the same

disadvantages with regard to the production of side-reactions as stilbœstrol. There are no figures available to show the comparative potency of this substance as compared with the others, on laboratory animals, and therefore it will merely be referred to from the clinical point of view.

Attempts to produce substances of the same degree of activity as the stilbœstrol series have not been particularly successful. The activity of diphenylethylene was shown to be definite, but slight. Robson and his colleagues[17] studied the activity of triphenylethylene (XIX) and have shown that derivatives in this series have activity, but again on a much lower plane than the stilbœstrol series. Robson and his colleagues[18,19] also made some interesting observations on halogen substituted derivatives of triphenylethylene. These substances have not aroused the interest of clinicians.



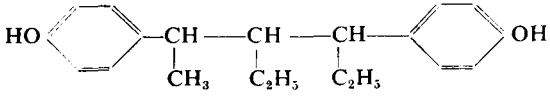
XVIII. Ethinyl œstradiol



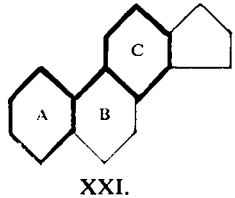
XIX. Triphenylethylene

Another modification of the stilbœstrol type of molecule was made by Blanchard and his colleagues[20,21] in the synthesis of octofollin, 2, 4-di(*p*-hydroxyphenyl)-3-ethyl hexane (XX). This is a derivative of hexane and the general resemblance to the stilbœstrol formula can be seen by comparing the formulæ. This substance is considerably less active than those of the stilbœstrol series, but it has been offered commercially and there are references to its activity in the human subject (Jaeger[22]).

In an attempt to explain the activity of synthetic œstrogens the author suggested (Dodds[23]) that some of the substances showing œstrogenic activity might be regarded as stages in the disintegration of the cyclopenteno-phenanthrene nucleus. For example, the activity of the diphenyl series might be explained by the opening of ring B in the manner shown in the diagram (XXI). With the discovery of stilbœstrol, however, this hypothesis was rather neglected, but recently it has been revived in a



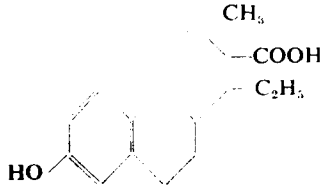
XX. Octofollin



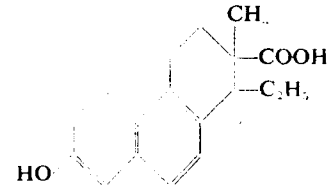
XXI.

very definite form, first by the striking work of Miescher and his colleagues, and later by Horeau and Jacques[24,25,26,27].

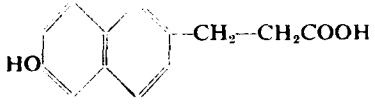
In 1933 Doisy and his colleagues[28,29] showed that it was possible to produce a very highly active substance from œstradiol by an oxidation process, but they failed to identify the compound so produced. The suggestion that highly active substances could be obtained in this manner suggested to Miescher and his colleagues that the disintegration of the œstrone molecule might produce substances of considerable activity. He therefore synthesised a number of compounds which corresponded to the œstrone molecule with the 5-membered ring opened. Two of these substances have shown great activity. These are referred to as doisynolic



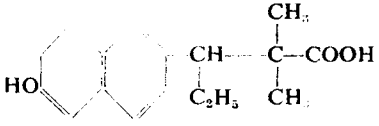
XXII. Doisynolic Acid



XXIII. *bis*Dehydrodoisynolic Acid



XXIV. Allenoic Acid



XXV. Dimethylethylallenolic Acid

acid and *bis*dehydrodoisynolic acid respectively (Miescher[24,25]) (XXII, XXIII). There have been extensive clinical trials with the 7-methyl derivative of *bis*dehydro-doisynolic acid. Miescher and his colleagues have published a number of papers on the synthesis of this compound and have also described[26] a shortening of the synthetic process, but even with this advantage the method of production is infinitely more costly than that of the simpler stilbene derivatives such as stilbœstrol.

Following up the disintegration idea still further, Horeau and Jacques[27] synthesised compounds which correspond to doisynolic acid with the 6-membered ring C opened. This yields a series of naphthalene derivatives, some of which have shown considerable activity. The parent substance has been called allenolic acid (XXIV) and the most active member of the series is dimethylethylallenolic acid (XXV), sometimes referred to as the Horeau acid.

Biological Activity

The biological standardisation of sex hormones and particularly of œstrogens has always presented very great difficulties. The œstrus reaction in the ovariectomised rat or mouse can only be treated quantitatively on a statistical basis, and the method proposed by Coward and Burn[30] is still the basis of all methods of standardisation. This in brief consists in an estimation of the amount of material required to produce full œstrus response in 50 per cent. of a group of ovariectomised animals. According to the size of the group, so vary the reproducibility and

accuracy of the result. To obtain a reproducibility of 20 per cent. some 20 animals must be used, and in order to get down to 10 per cent. 100 will be required. It can be seen that such a standardisation is very laborious, and in order to make the results of one laboratory comparable with those of another the League of Nations Committee on Biological Standards introduced the international standards of œstrone and œstradiol some years before the war. With the isolation and characterisation of the pure compounds, the difficulties of standardisation very largely disappeared and the dubious use of "rat units" fortunately disappeared from the literature.

The introduction of synthetic œstrogens raises a whole series of new difficulties, and the comparison of the potency of the various synthetic and artificial œstrogens becomes an impossibility. In the first instance, it will be remembered that the potency of an œstrogenic substance depends not only on the actual weight of material administered, but on the length of time over which the administration is spread. By and large one can say that the more one fractionates the dose, the greater will be the potency shown. One of the great difficulties with the synthetic œstrogens is their rate of absorption and destruction in the animal body. It is therefore very difficult to compare on any sound basis the activity of, say, stilbœstrol as against œstradiol. Again, the sensitivity of animals varies from laboratory to laboratory, and therefore it is impossible to compare potencies arrived at in one laboratory with those of another. In the Courtauld Institute a method of standardisation has been worked out, using ovariectomised rats and fractionated injections in sesame oil. By the use of this method it has been possible to arrive at the relative potency of the various synthetic substances which led to stilbœstrol. The results are only comparable in the one institution, and therefore it is not proposed here to make any suggestion that the potencies given are in any way absolute. By our method the following Table gives the potency of the synthetic œstrogens mentioned:

Substance	*Dose per Rat*
Stilbœstrol	0·3 to 0·4 μg.
Hexœstrol	0·2 μg.
Dienœstrol	0·4 μg.
*7-methyl-*bis*dehydro-doisynolic acid	0·5 μg.
Dimethylethylallenolic acid	3·0 to 4·0 μg.

* This is the figure obtained for the racemic compound. Miescher[24] has resolved this and has found that the dextro (+) compound is active in rats by single subcutaneous injection in oil in dose of 10·0 μg., whereas the lævo (−) compound is active in rats in a dose of 0·05 μg.

From this table it can be seen that by the methods employed, hexœstrol is the most potent substance of the series when administered by subcutaneous injection in oil. With regard to oral administration, it would

appear that in the rat the most potent substance is 7-methyl-*bis*dehydrodoisynolic acid, with stilbœstrol occupying second place.

Clinical Activity

The earliest tests of stilbœstrol were made under the ægis of the Medical Research Council in 1939 (Bishop, Boycott and Zuckerman[31], Winterton and MacGregor[32]). Since that time a vast literature has accumulated describing the testing and dosage of the various synthetic œstrogens. Out of this very extensive, and in many cases highly uncritical, literature a number of facts emerge:

1. That the synthetic œstrogens are active in the human subject by mouth, and that they are efficient in the treatment of the various gynæcological disorders.

2. That all products in a varying degree do cause side reactions, varying from slight nausea to, very rarely, severe symptoms such as vomiting, skin rashes, and so forth. By and large the reactions are never so severe as to necessitate the discontinuance of the treatment.

3. Astonishing diversity of opinion occurs on the relative potency and percentage of side reactions in these various compounds. In the first instance, the question of side reactions has been the subject of much speculation. Various groups of workers have claimed that one of the synthetic œstrogens is much less prone to produce side reactions than another, and from this it has been concluded that the toxicity is due to some peculiarity of the molecule. To the present writer this has always seemed an unlikely explanation, and in view of the fact that it is possible to get the same type of side reactions with compounds of such widely different structure as ethinyl œstradiol, doisynolic acid and allenolic acid, stilbœstrol, and so forth, it would appear much more reasonable to suppose that the toxicity is associated with the œstrogenic potency. It is known that the naturally-occurring œstrogens are rapidly destroyed in the body, whereas most of the synthetic œstrogens are excreted in the urine either unchanged in part or in conjugation with glucuronic acid.

It has been usual to assume that the sensitivity of all mammals is roughly the same for œstrogens, but there is now considerable evidence that such is not the case, and that it is most unwise to assume that the human female will react in the same way as the laboratory animals. The difficulty in the past has been the lack of any quantitative work on the subject. It is therefore with very great interest that the paper by Bishop, Kennedy and Wynn-Williams[33] has been received. These authors, recognising the lack of quantitative data, have attempted to standardise the œstrogens on the human subject by using œstrogen withdrawal bleeding as a criterion. If œstrogens are given to a menopausal woman with amenorrhœa, amelioration of the symptoms of the menopause occurs almost immediately. If, after a fortnight or so, treatment is suddenly stopped, a small vaginal hæmorrhage occurs. This has been termed œstrogen withdrawal bleeding. Bishop and his colleagues have used

this as an end-point in their standardisation, and by determining the minimum amount of orally active œstrogen necessary to induce this phenomenon, have been able to place the compounds tested in order of potency. The result was that, of the substances tested, stilbœstrol is the most potent. In view of the extremely important nature of their conclusions, the summary is quoted *in extenso:*

> " A method is described for comparing the potency of œstrogens in man. It consists in giving the œstrogen daily by mouth in 14-day courses to amenorrhœic women and recording whether œstrogen withdrawal bleeding takes place.
>
> " The results obtained indicate that dienœstrol is about a quarter, doisynolic acid about a fifth, and hexœstrol about an eighteenth as potent as stilbœstrol.
>
> " Investigation of the incidence of ' toxicity ' indicates that stilbœstrol is more likely to produce nausea in therapeutic doses than are dienœstrol, doisynolic acid and hexœstrol.
>
> " Reasons are given for choosing this end-point, and for the failure to devise any other suitable method of assessment at different levels of œstrogenic response, such as the relief of menopausal symptoms, the production of an œstrous vaginal smear, and the suppression of lactation."

This work is of very great interest in that it shows the folly of applying results obtained on animals to the human being. For example, there appears to be little doubt that 7-methyl-*bis*dehydro-doisynolic acid is highly potent in the rat and mouse by mouth, yet it appears from the results of Bishop and his colleagues to be relatively impotent in the human female.

Finally, ethinyl œstradiol has been the subject of a number of publications, and there is no doubt that it is able to replace the naturally-occurring œstrogens in the same way as stilbœstrol. A number of papers have appeared in America which show that menopausal symptoms can generally be controlled by daily doses of 0·05 to about 0·3 mg. (Wiesbader and Fillet[34], Groper and Biskind[35], Salmon *et al.*[36]). Birnberg *et al.*[37] have used ethinyl œstradiol with success for the treatment of the menopause and of amenorrhœa, for the suppression of lactation and for the induction or hastening of labour. Ethinyl œstradiol can also be used, like other œstrogens, for the treatment of carcinoma of the prostate (McCrea[38]). Some papers have appeared in which the potency of ethinyl œstradiol is compared with that of other œstrogens, natural and artificial. Harding[39] in a series of 47 cases used ethinyl œstradiol and other œstrogens to treat hypo-ovarian symptoms. Ethinyl œstradiol was shown to be the most active of the substances used, but like stilbœstrol was liable to cause " mild toxic reactions." Jeffcoate *et al.*[40] have compared ethinyl œstradiol with other œstrogens on its power to suppress lactation. By this criterion it is also shown to be the most

active. However, as pointed out by Bishop *et al.*[35], this is "an unsuitable method for the clinical assessment of œstrogens," and it is not one that lends itself to quantitative consideration. Finally, Soule[41] has compared ethinyl œstradiol, using œstrogen withdrawal bleeding as the endpoint, with stilbœstrol, α-œstradiol and "mixed œstrogens." The various œstrogens were only tested on 1 patient, so the results can hardly be considered as statistically significant, but it was shown in this case that ethinyl œstradiol was the most active, producing œstrogen withdrawal bleeding with a dose of 0·05 mg. per day for 21 days, as against a dose of 4 mg. of stilbœstrol for 13 days. Both these substances caused nausea when used in effective dosage.

Future Research

The success obtained in the field of synthetic œstrogens leads one to speculate as to future possibilities in the extension of research. Whilst it is always unwise to prophesy, there would appear to be two main lines of work.

Firstly, is it possible to synthesise compounds with a more selective action on the various tissues acted upon by œstrogens? As Parkes[42] has pointed out, the word œstrogen is rather an unfortunate one, since it focusses attention on only one aspect of these compounds' activities, namely, the production of œstrus changes in the vagina. He has suggested that the term "gynæcogenic" would be better, as this would include all the various activities associated with œstrogenic power, such as development of secondary sexual characteristics, action upon the uterus, breast and anterior lobe of the pituitary. Many have speculated as to whether it would be possible to synthesise a substance with selective action on the anterior lobe of the pituitary, whilst at the same time having little action on the breast, uterus and so on. The advantage of such a compound in the treatment of carcinoma of the prostate is obvious. It is the writer's opinion that there is no evidence that such compound could be found. Experience suggests that these compounds act in their entirety, and that it is not possible to segregate or separate the various actions. In other words, the results are due to œstrogenic activity *per se*.

The second line of speculation is whether it will be possible in the future to make synthetic analogues of the other steroid hormones. In other words, would it be possible to produce a compound with, let us say, androgenic activity, which bears no more resemblance to testosterone than does stilbœstrol to œstradiol. Whilst on general grounds it would seem possible that such compounds could be produced, until one has actually been synthesised and its action demonstrated it is obviously idle to speculate.

In conclusion it may be stated that the clinician has a wide selection of artificial œstrogens from which to choose to treat his patients by the oral route. Again, consideration of the evidence would seem to indicate that there is little to choose between any of these substances, and that they are likely to produce side reactions in direct proportion to their œstrogenic potency.

REFERENCES

1. Domagk, *Dtsch. med. Wochr.*, 1935, **61,** 250.
2. Trefouel, Trefouel, Nitti and Bovet, *C. R. Soc. Biol. Paris*, 1935, **120,** 756.
3. Stockard and Papanicolaou, *Amer. J. Anat.*, 1917, **22,** 225.
4. Cook, Dodds and Hewett, *Nature*, 1933, **131,** 56.
5. Cook and Dodds, *Nature*, 1933, **131,** 205.
6. Cook, Dodds, Hewett and Lawson, *Proc. roy. Soc. B.*, 1934, **114,** 272.
7. Dodds and Lawson, *Nature*, 1936, **137,** 996.
8. Dodds and Lawson, *ibid*, 1937, **139,** 627.
9. Campbell, Dodds and Lawson, *Nature*, 1938, **141,** 78.
10. Dodds, Golberg, Lawson and Robinson, *Nature*, 1938, **141,** 274.
11. Campbell, Dodds and Lawson, *Nature*, 1938, **142,** 1121.
12. Dodds, Golberg, Lawson and Robinson, *Nature*, 1938, **142,** 34.
13. Huggins, Scott and Hodges, *J. Urol.*, 1941, **46,** 997.
14. Horeau, *Trans. 8° Congres de Chimie Biologique, Paris*, 1948 (in press).
15. Anner and Miescher, *Experimentia*, 1948, **4,** 251.
16. Inhoffen and Hohlweg, *Naturwiss.*, 1938, **26,** 96.
17. Robson and Schonberg, *Nature*, 1937, **140,** 196.
18. Robson and Schonberg, *Nature*, 1942, **150,** 22.
19. Robson and Fahim, *Nature*, 1938, **142,** 292.
20. Blanchard, Stuard and Tallman, *Endocrinol.*, 1943, **32,** 307.
21. Council on Pharmacy Report, *J. Amer. med. Ass.*, 1944, **124,** 647.
22. Jaeger, *J. Indiana State med. Ass.*, 1944, **37,** 117.
23. Dodds, *Helv. chim. Acta*, 1936, **19,** 49.
24. Miescher, *et al.*, *Helv. chim. Acta*, 1944, **27,** 1727.
25. Heer, Billeter and Miescher, *Helv. chim. Acta*, 1945, **28,** 991.
26. Miescher, *Helv. chim. Acta*, 1946, **29,** 586, 1231.
27. Horeau and Jacques, *C.R. Acad. Sci., Paris*, 1947, **224,** 862.
28. MacCorquodale, Levin, Thayer and Doisy, *J. biol. Chem.*, 1933, **101,** 753.
29. MacCorquodale, Thayer and Doisy, *J. biol. Chem.*, 1933, **99,** 327.
30. Coward and Burn, *J. Physiol.*, 1927, **63,** 270.
31. Bishop, Boycott and Zuckerman, *Lancet*, 1939, **236,** 5.
32. Winterton and MacGregor, *Brit. med. J.*, 1939, **1,** 10.
33. Bishop, Kennedy and Wynn-Williams, *Lancet*, 1948, **255,** 764.
34. Wiesbader and Filler, *Amer. J. Obstet. Gynec.*, 1946, **51,** 75.
35. Groper and Biskind, *J. clin. Endocrinol.*, 1942, **2,** 703.
36. Salmon, Geist, Walters and Mintz, *J. clin. Endocrinol.*, 1941, **1,** 556.
37. Birnberg, Livingston, Kirzok and Sherber, *Amer. J. Obstet. Gynec.*, 1947, **54,** 885.
38. McCrea, *J. Urol.*, 1946, **56,** 697.
39. Harding, *Amer. J. Obstet. Gynec.*, 1944, **48,** 181.
40. Jeffcoate, Lister, Hargreaves and Roberts, *Brit. med. J.*, 1948, **2,** 809.
41. Soule, *Amer. J. Obstet. Gynec.*, 1942, **44,** 684.
42. Parkes, *Nature*, 1938, **141,** 36.