Chemical Structure in relationship to Hormone and Biological Activity

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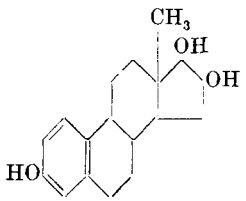
(15. VI. 36.)

Since the commencement of synthetic organic chemistry in the last century the chemist and pharmacologist have striven continuously to correlate chemical structure with biological activity. The early successes in this field were undoubtedly obtained on the purely pharmacological side and it was not until the establishment of the principle of internal secretion that the organic chemist really became interested in biologically active principles or hormones, as they are now called. Whilst the first clearcut example of the functions of an endocrine organ was obtained in the case of the thyroid, the actual chemical solution to that problem was not forthcoming until some twenty-five years after the first use of thyroid extract. The demonstration of the pressor activity of the extract of the suprarenal gland by *Oliver* and *Schafer* in 1895 was quickly followed by the isolation of adrenalin in crystalline form by *Abel* and other workers and later the synthesis by *Stolz* rounded off this second hormone problem. That the most dramatic and unexpected developments have occurred during the last ten years in work on the sex hormones will not be questioned by anyone. It is only necessary to recapitulate very briefly the now well-known story of the early work on the sex hormones. The first clear-cut example of replacement therapy following removal of the ovary was given by the work of *Herrmann* and *Fränkel* in 1915. These workers proved that the cyclical phenomena of heat could be reproduced in a castrated female animal by the injection of an alcoholic extract of the ovary of another animal. They showed that the compound can be obtained from ovaries, is soluble in ether and acetone, and that it only contained carbon, hydrogen and oxygen. Owing to the difficulty of converting their method of standardisation from a qualitative one to a quantitative one very little progress was made in the chemistry of the active principle. It was not until *Allen* and *Doisy*'s application of the *Stockard Papanicolaou* method of vaginal smears to the problem that any progress could be made. Numerous difficulties were ex-

perienced in purifying the material and these were not overcome until *Aschheim* and *Zondek* discovered the presence of the hormone in the urine of pregnancy. This, at the same time, gave a large supply of material and also presented it in a form more suitable for chemical purification than had hitherto been found. The crystallisation of the hormone by *Doisy* in America and *Butenandt* in Germany simultaneously in 1929 gave the organic chemist the opportunity of determining its structure. It is not necessary for me to describe the various researches which led to the adopting of the formulae for the oestrogenic active substances. These are five in number up to the present time and their formula is shown:—



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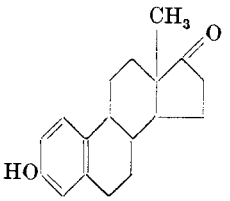

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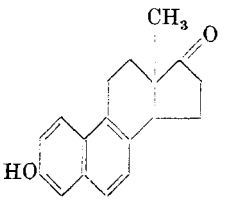

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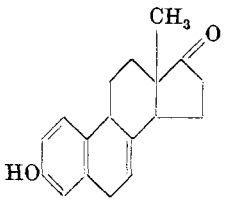

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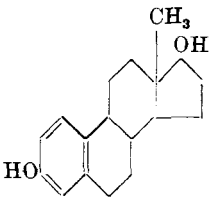


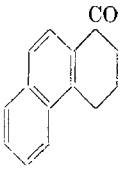
(5)

1. oestriol, 2. oestrone, 3. equilenin, 4. equilene, 5. oestradiol

The fact that at least five compounds have been isolated possessing oestrogenic activity indicates that either the oestrus mechanism does not demand the specificity that has always been associated with complex biological reactions, or that these reactions in general are nothing like so specific as we have hitherto supposed. It is only comparatively recently that this problem has received any sustained attention. It is my purpose in this lecture to develop in so far as is possible what I hope will be new views on these problems.

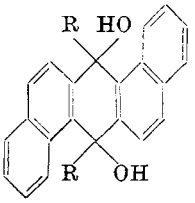
The first oestrogenic compounds isolated in a pure state were oestrone and oestriol and this led to the speculation as to whether other substances apart from these complex ones would be capable of producing oestrus. The possibility of oestrogenic activity in a series of phenanthrene compounds was carefully explored in 1932 and 1933 *(Cook, Dodds, Hewett* and *Lawson)*. The very surprising

observation was made that 1 keto-1:2:3:4-tetrahydrophenanthrene pcssesses all the known biological activities of oestrone and oestriol.



1-keto-1:2:3:4-tetrahydrophenanthrene

This work was continued and a whole series of phenanthrene containing compounds were proved to be active. The most interesting series of compounds were the dialkyl-dibenzanthracene diols:—



It will perhaps be in order to digress for a moment on the biological activity of these compounds as it is essential to realise that although they differ so markedly in constitution from the oestrone molecule, they do possess all the known actions of oestrone. Thus they will induce both vaginal and uterine changes in the castrated animal and will also cause premature puberty in the infantile mouse or rat. This is demonstrated by the opening of the vagina which, it will be remembered, is represented as a solid cord of cells in the immature animal. Perhaps the most striking demonstration to those not familiar with the physiology of the rodents is the effect of these compounds on the plumage of birds. It has been known for a considerable time that oestrone is capable of reversing the plumage from male to female in the leghorn capon. The following illustration (plate I) indicates the effect of the injection of 1-keto-1:2:3:4-tetrahydrophenanthrene into such capons. It will be seen that a complete reversal of the plumage occurs under the influence of this relatively simple molecule.

Furthermore, it has been shown by *Wolfe* that the di-*n*-propyldiol referred to previously is capable of preventing the appearance of castration cells in the anterior lobe of the pituitary in precisely the same manner as can be obtained by oestrone. *Hemmingsen* has also demonstrated that this substance can confer on castrated female animals the maternal and mating instinct. It has also been shown, *(Cook, Dodds,* and *Lawson)* that this substance can be used in place of oestrone for producing the characteristic progestational uterine changes which occur during the administration of progesterone and

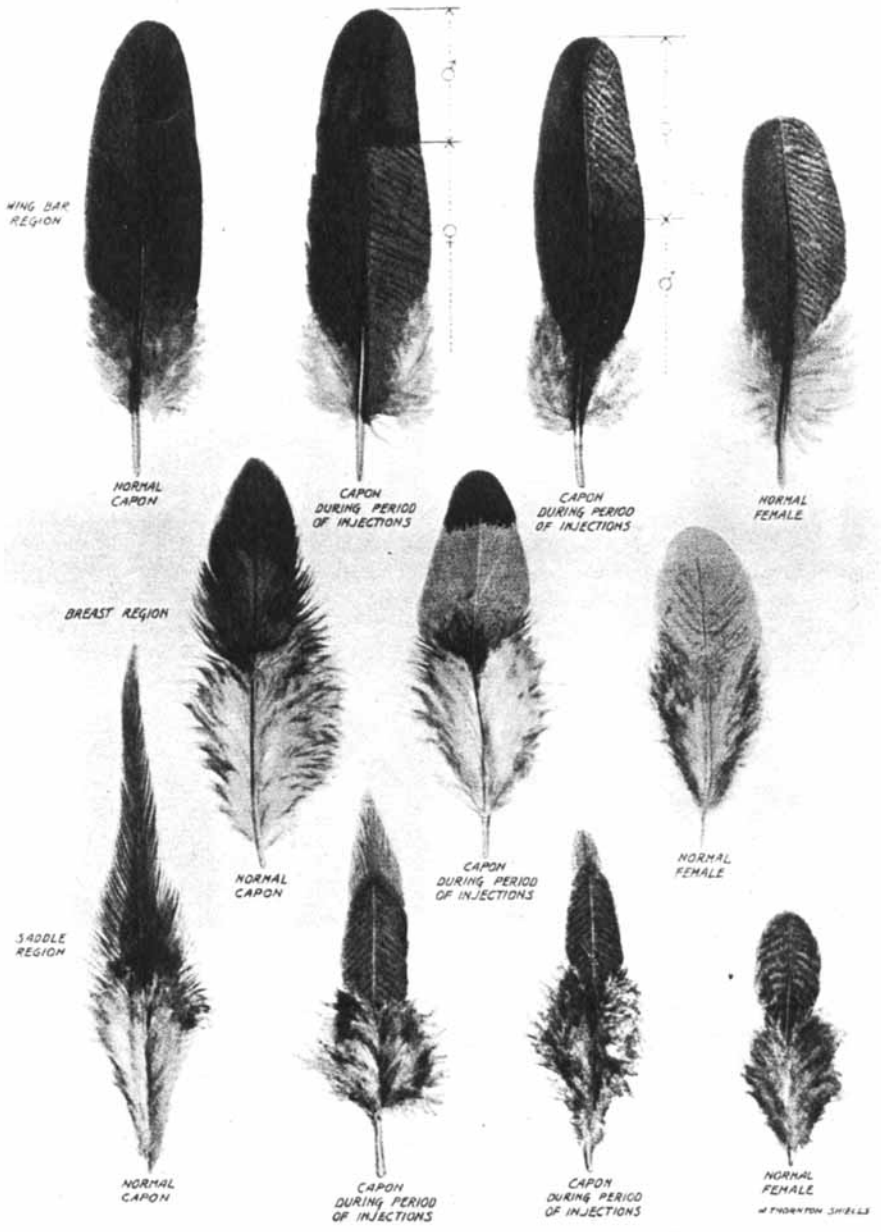
oestrone to a castrated rabbit. We may safely conclude, therefore, that these compounds reproduce the whole of the action of oestrone and that they will stimulate the same mechanism as oestrone. It is very important indeed to make this point as clear as possible since serious mistakes can be made if only a superficial resemblance of activity were demanded. As an illustration of this may be quoted the well-known action of thyroxine. If thyroxine be administered to a normal subject or to a myxoedematous person an increase in the basal metabolic rate results. The same reaction can be elicited by 3:5-di-iodo-thyronine and in the use of both agents myxoedema can be cured. Now if only a superficial examination is made it would appear that the polynitrophenols 2:4-dinitro-phenol and 4:6-dinitro-o-cresol are capable of simulating the action of thyroxine. Both these substances will increase the basal metabolic rate of the normal and myxoedematous subject, but they will not cure myxoedema as shown by the following photographs (plate II) of a patient treated (a) with dinitro-o-cresol and (b) thyroxine. The basal metaboliic rate in the case of the dinitro-o-cresol was increased to +20 and it can be seen that the myxoedema is unrelieved. In the case of the treatment with thyroid extract although the basal metabolic rate is only +10 the myxoedema has completely disappeared.

Moreover it has been shown that dinitro-o-cresol stimulates the metabolism of the isolated tissue slice, whereas this is an action which cannot be obtained with thyroxine. It is, therefore, essential in the study of oestrogenic agents to make quite sure that they are capable of reproducing every part of the oestrus cycle and this has been proved by the experiments quoted above. It would appear that the activity of the synthetic compounds differs from that of oestrone only in the qualitative sense. The relative potency of some of the dibenzanthracenealkyl diols is indicated in the following tables (p. E 53).

In a paper now in the press *(Cook, Dodds,* and *Lawson)*, the activity of a number of other substituents of this series of diols is described. Thus where R is the *iso*-propyl group the activity is reduced to 0.25 of a mg., that is about one-tenth of the corresponding di-*n*-propyl compound. The *iso*-butyl compound is only about one-tenth as active as the di-*n*-butyl. It is interesting to note that the *cyclo*pentyl derivative possesses quite strong activity, where as the corresponding known amyl compound is inactive. Where R is the allyl or *cyclo*hexyl group an inactive compound results.

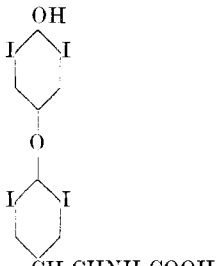
If we review the chemical structure of these compounds and we search for some common point in their structure we are left with only one, namely the phenanthrene nucleus which is common to all of them. Until comparatively recently it was the common opinion

Plate I.

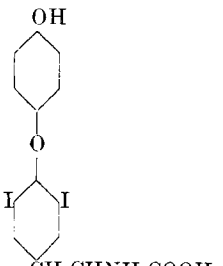


[Proc. Roy. Soc.]

Effects of injecting 1-keto-1:2:3:4-tetrahydrophenanthrene on capon plumage.



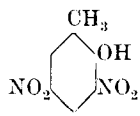
Thyroxine



3′5′ di-iodo-thyronine

(a) Patient treated with dinitro-o-cresol.
B. M. R. +20

(b) Patient treated with thyroxine.
B. M. R. +10



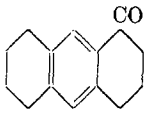
4:6-dinitro-o-cresol.

[*Dodds* and *Robertson*]

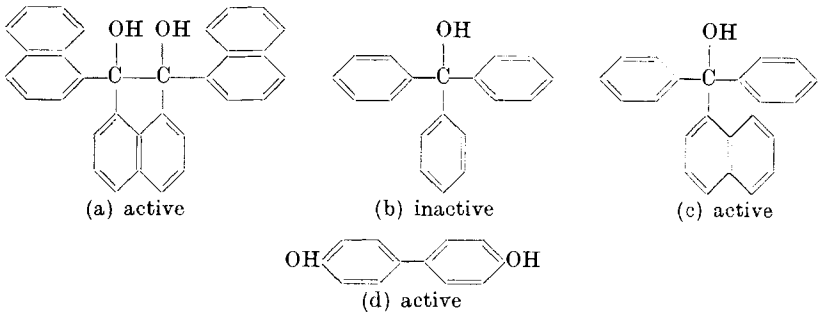
Substance	No. of Animals	Dose in mg.	Per cent Positive	Remarks
Dimethyl	Rats 5	100	Nil	—
Diethyl	,, 5	100	100	Oestrus lasted 13 days
	,, 5	10	100	,, ,, 13 ,,
	,, 5	1.0	100	,, ,, 6 ,,
Di-*n*-propyl	,, 5	5	100	—
	,, 5	1.0	100	—
	,, 5	0.1	60	—
	,, 10	0.105	80	—
	,, 10	0.025	40	—
Di-*n*-butyl	,, 10	100	100	Oestrus lasted 28 days Animals then killed.
	,, 9	1.0	50	Oestrus lasted 48 hours
	,, 14	0.1	50	,, ,, 36 ,,
	Mice 11	1.0	100	Animals in oestrus when killed on 10th day
	Rats 10	10	90	Suspension in water
	,, 10	1.0	10	per os.
Di-*n*-amyl	,, 5	100	Nil	—
Di-*n*-hexyl	,, 3	100	,,	—
1-Keto-1:2:	Rats 36	100	100	Oestrus lasted 20 days
3:4-tetra-hydrophenan-threne	,, 5	100	Nil	Suspension in water administered per os.

of workers in this field that oestrogenic activity demanded the presence of this ring system. Intensive research has been undertaken in my department in conjunction with *W. Lawson* in attempts to simplify the molecule. It can readily be seen that any attempt to postulate a theory of structure in relation to function in the compounds already discussed would be fraught with the greatest difficulties since not only would the question of mere structure be involved, but the infinitely more complicated one of stereo-chemical relations of the groups would have to be considered.

Before entering this difficult field it was decided to explore many types of compounds with a view to seeing whether any substance without the phenanthrene nucleus would be active. An interesting compound from the physiological point of view was described by us *(Cook, Dodds, Lawson* and *Hewett)* namely 1-keto-1:2:3:4:5:6:7:8-octahydroanthracene. This substance when injected into ovariecto-



mised rats would produce only pro-oestrus and is incapable of producing the full cornification necessary before a compound can really be deemed oestrogenic. From this it would appear that certain activity on the genital tract could be obtained by substances not possessing the phenanthrene nucleus.



Types of synthetic oestrogenic compounds not containing the phenanthrene nucleus.

Furthermore, it was also shown by us that calciferol in big doses possesses a feeble oestrogenic activity. If the present formula of this substance be considered it can be seen that it does not possess the phenanthrene structure since ring two has been opened by the process of irradiation of ergosterol. The first active substance that we were able to obtain which did not possess the phenanthrene nucleus was 1:2-dihydroxy-1:2-di-α naphthyl-acenaphthene (a). This substance is very powerfully oestrogenic and if 100 mgs. be administered to a rat it will remain in oestrus for a period of 40 days. It is also capable of causing the change in the feathers described after administration of other synthetic oestrogenic agents and oestrone. The substance is active in very much smaller doses than 100 mgs. and appears to be capable of maintaining an animal in full oestrus for a very prolonged period. In an attempt still further to simplify the molecule a simple compound, namely triphenyl carbinol (b) was injected, this, however, was found to be inactive. In view of the activity of the α-naphthyl-acenaphthene compound referred to above diphenyl-α-naphthyl carbinol (c) was investigated and this was found to give a definite oestrus response. The next stage in the research consisted in a study of various derivatives of di-p-hydroxyphenylmethane and we were successful in producing oestrus with a number of di-p-hydroxyphenyl methanes. The activity of these compounds is given in the following table (p. E 55).

Up to the present the simplest substance which is capable of giving a full oestrus response is 4:4′-dihydroxydiphenyl (d). Experiments are in progress to study the effects of modifying the positions of the hydroxyl groups and also of increasing the length of the carbon chain joining the two p-hydroxy-phenyl parts of the

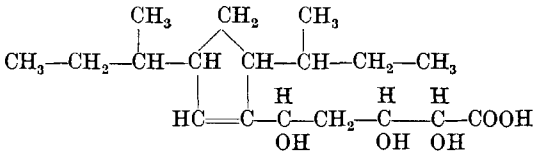
Substance	Dose in mg.	Percentage Positive
1:2-Dihydroxy-1:2-di-α-naphthyl-acenaphthene	100	100*
1:2-Dihydroxy-1:2-di-α-naphthyl-acenaphthene	10	100
1:1-Di-α-naphthyl-acenaphthenone	100	100
α-Naphthyl benzoin	100	40
Diphenyl-α-naphthyl glycol	100	60
Diphenyl-α-naphthyl carbinol	100	100
4-4-Dihydroxy-diphenyl methane	100	100
Di-(p-Hydroxyphenyl)dimethyl methane	100	100
Di-(p-Hydroxyphenyl) methyl ethyl methane	100	100
Di-(p-Hydroxyphenyl) methyl propyl methane	100	100
Di-(4-Hydroxy-3-methyl phenyl)di methyl methane. . .	100	100
Di-(4-Hydroxy-3-methyl phenyl)-1:1-cyclohexane . . .	100	100
2:4-Dihydroxy-triphenyl methane carboxylic acid lactone	100	100
$4:4^1$-Dihydroxy benzophenone	100	60
$4:4^1$-Dihydroxy diphenyl	100	100

* Rats remained in oestrus 40 days.

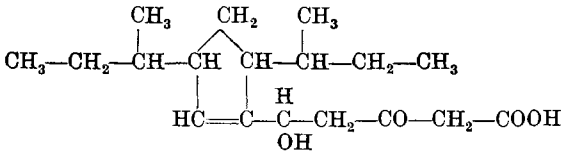
molecule together. It can be definitely stated at the present time that the introduction of the double bond into this chain materially increases the activity.

The question that naturally arises is the significance of these rather unusual and certainly unexpected observations. Does it mean that the oestrus mechanism is relatively unspecific compared with other biological processes? In the opinion of the speaker this is not necessarily the case and it must be most emphatically pointed out that whilst at the present time we are unable to formulate any theory which will explain the activity of all these compounds this does not mean that oestrus can be produced in an ovariectomised animal provided a sufficient quantity of any hydroxyphenyl or phenanthrene compound be given. This is clearly indicated by our negative results which I do not propose to burden you with. A study of the dibenzanthracenealkyl diols shows that a very small change in the alkyl side chain will result in an inactivation of the molecule. For example, whilst the di-*n*-propyl compound is roughly of the same order of activity as oestriol, the corresponding di-allyl compound is inactive when given in amounts corresponding to forty thousand times the dose. Again an alteration of the position of the hydroxyl groups in the diphenyl compound will result in inactivation. It must also be borne in mind that no other biological reaction has been so intensively studied from this point of view as the oestrus cycle and therefore we have no knowledge as to the specificity of the other biological processes. What available informa-

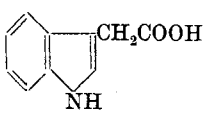
tion we have would seem to indicate that they also are nothing like so specific in their demands as we have hitherto been led to believe. Take for example the case of the male sex hormone. It is well known that at least four substances, namely androsterone, dehydroandrosterone, testosterone and androstandiol are capable of producing the changes associated with male sex hormone activity. The case of the thyroid gland has already been referred to where both thyroxine and 3:5-di-iodo-thyronine are capable of curing myxoedema and raising the basal metabolic rate. In the case of the plant hormones *Kögl* has shown that auxine is active in two forms, auxine a and auxine b:—



Auxine a

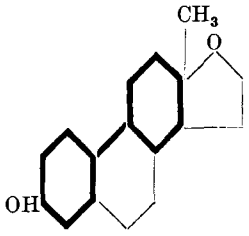


Auxine b



Indole-acetic acid

whilst a substance of entirely different chemical constitution namely indole-acetic acid is also very potent in producing the auxine phenomena. That there must be some underlying principles in this work seems to be essential, yet at the present time it would appear to be obscure. One possibility that may be borne in mind is that the oestrone molecule may be broken down in the body to relatively simple substances which might be the true oestrogenic agent. For example, one could imagine that the oestrone molecule might break down according to the following scheme which would provide a compound of the diphenyl type.



It must, however, be clearly stated that this is pure speculation and that no evidence exists at the present time of such a supposition.

It may well be that the active substances are relatively simple break-down products of complicated molecules and that the continuation of this work may succeed in producing substances of simple constitution of an even higher order of activity.

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Bibliography.

The following general articles will be found to contain references to all the work described in this lecture:—

Dodds, E. C., The Goulstonian Lectures 1934. Lancet i **1934**, 931, 987 and 1048.

Dodds, E. C., The Harvey Lectures, 1934—1935.

Dodds, E. C., Ergebnisse der Physiologie **37**, 264 (1935).

Dodds, E. C., and *Dickens, F.*, The Chemical and Physiological Properties of the Internal Secretions, London 1925.

Abel, J. J., Johns Hopkins Hosp. Bull. **9**, 215—218 (1898). Z. physiol. Ch. **28**, 318—362 (1899). Johns Hopkins Hosp. Bull. **12**, 337—343 (1901). Ibid. **13**, 29—36 (1902). Proc. Amer. Physiol. Soc. **29** (1902); Amer. J. Physiol. **8**. Ibid. **30** (1902); Amer. J. Physiol. **8**. Ibid. **31** (1902); Amer. J. Physiol. **8**.

Allen, E., and *Doisy, E. A.*, J. Amer. Med. Assoc. **81**, 819 (1923).

Aschheim, S., and *Zondek, B.*, Klin. Wochschr. **6**, 1322 (1927).

Butenandt, A. (1929): Naturwiss. **17**, 879 (1929). (1931): Z. angew. Ch. **44**, 905 (1931). (1932): Nature **130**, 238 (1932). (1933): Naturwiss. **21**, 49 (1933). (1935): Dtsch. med. Wochschr. **1935**, i, 781.

Cook, J. W., Dodds, E. C., Hewett, C. L., Nature **131**, 56 (1933).

Cook, J. W., Dodds, E. C., Hewett, C. L., Lawson, W., Proc. Roy. Soc. **114** [B], 272 (1934).

Cook, J. W., Dodds, E. C., and *Lawson, W.*, ibid. Sept. 1936.

Dodds, E. C., Lawson, W., Nature **137**, 996 (1936).

Doisy, E. A., Veler, C. D., and *Thayer, S. A.*, Amer. J. Physiol. **90**, 329 (1929). J. Biol. Chem. **86**, 499 (1930).

Hemmingsen, A. M., 1935. Proc. International Phys. Cong. Moscow.

Hermann, E., Monatsschr. f. Geburtsh. u. Gynäk. **41**, 1—50 (1915).

Kögl, F., B. **68** A, 16 (1935).

Oliver, G., and *Schafer, E. A.*, J. Physiol. **18**, 277 (1895).

Stolz, 1904, D.R.P., Farbw. vorm. Meister Lucius und Brüning, Klasse 12 q, 152 814 155 652 and 157 300.

Wolfe, J. M., Amer. J. Physiol. **115**, 3, May (1936).