nium at temperatures up to 350°, but no phenanthrenes could be isolated from the fluorescent oily products.

Further, the formation of these spiranes was suspected by us, and their presence in the reaction products predicted, because of our experience with the indanes and ionenes.[2]

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THE OESTROGENIC ACTIVITY OF CERTAIN PHENANTHRENE AND HYDROPHENANTHRENE DERIVATIVES

A SOMEWHAT novel technique of bio-assay has been developed in the course of an investigation of the series of phenanthrene and hydrophenanthrene derivatives listed in Table I. The synthetic substances were prepared by Fieser and his collaborators, as indicated in the preceding note,* and their values for the melting points are included in the table.[1,2,3,4]

The oestrogenic activity of the compounds was tested by the intraperitoneal injection of sesame oil solutions into spayed mice at two weeks after ovariectomy. Intraperitoneal injections were employed in order to insure absorption of the oily solutions, since it has been shown[5] that such solutions are retained subcutaneously for long periods. Furthermore, we have observed definite inflammatory reactions against subcutaneous injections of certain of these and related compounds of such a nature that the oily material is eventually walled off by connective tissue overgrowth. There are in addition obvious lymphatic reactions against the subcutaneously injected material.

Various methods of assay were tested, and a standard procedure was evolved, as follows: two intraperitoneal injections of 0.2 cc each were made at twelve-hour intervals and the first vaginal smear was taken at the time of the second injection; at approximately twelve-hour intervals thereafter vaginal smears were taken until a total of nine readings was had on each animal. The animals were taken in groups of five so that a total of forty-five smears were recorded in a routine test. Prooestrus and oestrus smears were recorded as positive and the total positive count was compared with a set of control (sesame oil injected) animals. As our standard of comparison for degree of activity we employed a set of determinations on animals receiving various dosages of crystalline oestrone in sesame oil. The standard oestrone curve is given in Fig. 1. Injections of 0.1 γ and above give a statistically significant increase of positive smears over the control series.

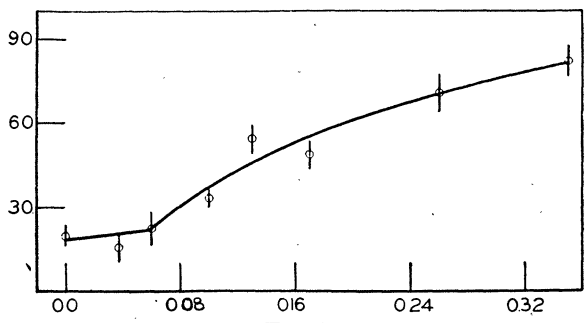

FIG. 1

Abscissa-dosage of oestrone in gamma.

Ordinate—per cent. of positive smears in test mice. The vertical lines at each point are equal to twice the standard error of the measurements.

The synthetic compounds were ordinarily tested in dosages of 100 γ or less, and their activity in terms of oestrone was determined from the standard curve (Table I, column 4). With few exceptions (substances II, III and X) those substances showing no activity in 100 γ dosages were not tested further. On the basis of vaginal cornification alone substances II and III showed an activity of about 10,000 to 15,000 mouse units per gram with low dosages, but no significant increase in activity with higher dosages. Using our standard criteria, however, both compounds were inactive in dosages up to 200 γ. Substance XV, on the other hand, by our method of assay showed an exact proportional increase when the minimum effective dosage was quadrupled.

It will be noted that the addition of auxiliary hydroxy and methoxy groups results in a definite increase in activity. Furthermore, the addition of these groups at either position 6 or 7 results in the same degree of enhancement (compare substances XIII, XIV and XVII) and the presence of these groups at both 6 and 7 (substances XV and XVI) results in approximately three times the activity of the compounds with single additions at these positions. Closure of the fourth ring in the 1,2 position (substance V) is about half as effective in enhancing activity as the hydroxy and methoxy additions. This is the position of the cyclopenteno-ring typical of the native oestrogenic hormones. When the ring is in the 3,4 position (substance VI) we do not obtain a corresponding activity.

That the assay procedure we have employed offers a more sensitive indication of activity than ordinary

[2] *Jour. Am. Chem. Soc.*, 56: 185, 248, 959, etc.

* L. F. Fieser and others, SCIENCE, 83: 2162, 558, 1936.

[1] L. F. Fieser and E. B. Hershberg, *Jour. Am. Chem. Soc.*, 57: 1851, 1935.

[2] L. F. Fieser, M. Fieser and E. B. Hershberg, unpublished work.

[3] L. F. Fieser and E. B. Hershberg, *Jour. Am. Chem. Soc.*, 57: 2192, 1935.

[4] L. F. Fieser and E. B. Hershberg, unpublished work.

[5] R. Deansley and A. S. Parkes, *Jour. Physiol.*, 78: 155, 1933.

TABLE I

No.	Compound Name	m.p., °C. corr.	Activity as per cent. of oestrone activity
I.	1-Keto-1, 2, 3, 4-tetrahydrophenanthrene	94–95	0.56
II.	3, 4-Dihydrophenanthrene-1, 2-dicarboxylic anhydride[1]	263.5–264.5	weak*
III.	Phenanthrene-1, 2-dicarboxylic anhydride[1]	311–312	weak*
IV.	Phenanthrene-3, 4-dicarboxylic anhydride[2]	253.5–254	Inactive in 100 γ doses
V.	1[1], 3[1]-Diketo-1, 2-cyclopenteno-phenanthrene[2]	Dec. 240–245	0.22
VI.	1[1], 3[1]-Diketo-3, 4-cyclopenteno-phenanthrene[2]	201.4–202	Inactive in 50 γ doses
VII.	2, 3-Dimethyl-1, 4, 9, 10, 11, 12-hexahydrophenanthrene-11, 12-dicarboxylic acid[3]	176–177	Inactive in 100 γ doses
VIII.	Anhydride of VII[3]	95–96	Inactive in 100 γ doses
IX.	Monomethyl ester of VII[3]	157–158	Inactive in 100 γ doses
X.	Dimethyl ester of VII[3]	93.5–94	Inactive in 1000 γ doses
XI.	2, 3-Dimethyl-1, 4, 11, 12, 13, 14-hexahydrochrysene-13, 14-dicarboxylic anhydride[3]	196–196.5	Inactive in 100 γ doses
XII.	9-Methoxyphenanthrene-1, 2-dicarboxylic anhydride	249–250	0.11
XIII.	6-Hydroxy-1, 2, 3, 4, 9, 10, 11, 12-octahydrophenanthrene-11, 12-dicarboxylic anhydride[4]	160–160.5	0.56
XIV.	Methyl ether of XIII[4]	159–159.5	0.48
XV.	6, 7-Dihydroxy-1, 2, 3, 4, 9, 10, 11, 12-octahydrophenanthrene-11, 12-dicarboxylic anhydride[4]	147.5–148.5	1.58
XVI.	Dimethyl ether of XV	146.5–147	1.47†
XVII.	6-Methyl-7-hydroxy-1, 2, 3, 4, 9, 10, 11, 12-octahydrophenanthrene-11, 12-dicarboxylic anhydride[4]	134.5–135.5	0.55

* See text.

† Estimated from data on effects of a single injection.

methods is indicated by our data on substance I. Butenandt[6] obtained positive results when 70 mgms of this substance were injected in a single subcutaneous dose into spayed mice. Cook, Dodds et al.[7] observed marked activity with 100 mgm doses in spayed rats. From the data of these experiments the minimum active dose is about 30 γ. Furthermore, substance XIII, which showed definite activity in 25 γ dosages, gave completely negative results upon subcutaneous injection in 100 γ doses.

And substance XV was inactive in 1 mgm subcutaneous dosages (3 injections in 12 hours), in spayed rats on the basis of vaginal cornification, whereas a 14 mgm dosage in a single subcutaneous injection gave a positive result in rats. It must be emphasized that the method of administration seems to be the all-important factor in determining activity (*cf.* Cook, Dodds *et al.*).

In addition to their oestrogenic effects, certain of these compounds have shown definite activity in the immature rat test, in the augmentation of ovarian growth in rats following FSH stimulation and in the sterilization of rabbits. The details of these experiments will be published elsewhere.

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PLANT GROWTH EFFECTS OF HETEROAUXIN APPLIED TO SOIL AND PLANTS

β-INDOLYL acetic acid (heteroauxin) in extremely dilute concentrations is known to function as a growth-promoting hormone in plants. The crystalline material was synthesized in this laboratory according to the method of Majima and Hoshino.[1] The indolyl acetic acid used had a melting point of 164–166° C., 8.04 per cent. nitrogen and a neutral equivalent of 176.3; the corresponding theoretical values being 165° C., 7.99 per cent. and 175.1, respectively. An aqueous solution, 1/15,000, was used to determine its effect on growth, rooting, proliferation and bending. Actively growing seedlings were used to detect the effect of heteroauxin during the grand period of growth. One hundred and eighteen day old potted stock seedlings having approximately equal stem lengths were divided into two series. To each pot of the first series 15 cc. of the indole acid solution was added. Each pot of the second series was treated only with an equal volume of water. All the plants were kept under greenhouse conditions and were watered daily throughout the experimental period.

The effect of the indole acid on stem elongation was manifest at the end of the first experimental day, and reached its maximum on the sixth day following its administration (Table I). Curling of the cotyledons on treated plants appeared at the end of the second day, but by the end of the sixth day this effect began to disappear and by the tenth day had disappeared

[6] A. Butenandt and G. Schramm, *Ber.*, 68 B: 2303, 1935.

[7] J. W. Cook, E. C. Dodds, C. L. Hewett and W. Lawson, *Proc. Roy. Soc. Lond.*, B 114: 272, 1934.

[1] Majima and Hoshino, *Berichte*, 58: 2042, 1924.