XCIX. THE ACTIVATION OF FEMALE SEX HORMONES

II. α -OESTRADIOL AND ITS DI-ESTERS

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(Received 7 February 1938)

In the preceding paper of this series [1938] it was shown how the effect of oestrone can be systematically increased by suitable esterification. In these investigations special attention was directed to the testing of larger doses and the control of the duration of their effect. We now report on the results obtained in a similar way with α -oestradiol and its di-esters¹. The effect of the 3- and 17-monoesters of oestradiol will be described in a later paper. It is possible to differentiate between symmetric and asymmetric di-esters of oestradiol according to whether the 2 acid radicals in the 3- and 17-positions are the same or of a different kind. The di-esters used for the investigations as well as some of their characteristics are listed in Table I.

Table I

α -Oestradiol di-esters	M.P.* or B.P.† ° C.	Oestrus test (rat)	
		Threshold value S_p oestradiol =0.4 γ	Threshold value S_o based on oestradiol =0.1 γ
Diacetate	127-128	0.5	0.1
3-Acetate-17-propionate	112.7-113	0.6	0.1
Dipropionate	104-105	0.75	0.2
Di- <i>n</i> -butyrate	64-65	3.5	0.9
Di- <i>isobutyrate</i>	100.5-101.5	6.0	1.5
Di- <i>n</i> -valerate	220-230 0.05 mm.	7.0	1.8
Di- <i>n</i> -hexanoate	220-240 0.0005 mm.	8.0	2.0
Di- <i>n</i> -octanoate	240 0.0005 mm.	10.0	2.5
Di- <i>n</i> -decanoate	260-265 0.0001 mm.	25.0	6.2
Dipalmitate	63-65	27.0	6.8
3-Benzoylate-17-acetate	172-173	4.0	1.0
3-Benzoylate-17-propionate	167-167.5	4.0	1.0
3-Benzoylate-17- <i>n</i> -butyrate	128.5-129	9.0	2.2
3-Benzoylate-17- <i>n</i> -valerate	133-133.5	17.0	4.2
17-Benzoylate-3-acetate	161-161.5	8.0	2.0
17-Benzoylate-3-propionate	165-166	28.0	7.0
17-Benzoylate-3- <i>n</i> -butyrate	141.5-142	45.0	11.2
Dibenzoate	171.6-172	125.0	36.2

* The melting-points are taken with a short thermometer.

† Bath temperature.

The esters include representatives of the purely aliphatic, the mixed aliphatic-aromatic and the purely aromatic series. Only the diacetate, the 3-benzoylate-17-acetate and the dibenzoate have been previously described. A report has been given elsewhere on the manufacture of eleven esters [Miescher & Scholz,

¹ As regards the preliminary communications in this field see the preceding paper p. 141, note 1.

1937]. Here we describe only those di-esters about which nothing has been published.

Oestradiol-3:17-di-n-hexanoate. A solution of 720 mg. of oestradiol in 4 ml. of pyridine was mixed with 1.06 g. *n*-hexanoyl chloride (3 mol.) and after 10–12 hr. it was heated to 70° for 30 min. Dilute acid was added and the oil which separated taken up in ether. The ethereal solution was washed, dried and filtered, and the solvent evaporated. The residue was an oil which was distilled twice at 0.0005 mm. (bath temperature 220–230°). All attempts to crystallize the distillate have failed. (Found: C, 76.33; H, 9.58 %. $C_{30}H_{44}O_4$ requires C, 76.86; H, 9.47 %.)

Oestradiol-3:17-di-n-octanoate. Oestradiol was dissolved in pyridine, mixed with 3 mol. *n*-octanoyl chloride and the reaction conducted as above. The oestradiol-3:17-di-*n*-octanoate obtained after the usual working up was oily and was purified by double distillation at 0.0005 mm. (bath temperature 240°). So far the yellow oil has not been crystallized. (Found: C, 77.88, 77.80; H, 10.26, 10.10 %. $C_{34}H_{52}O_4$ requires C, 77.99; H, 10.00 %.)

Oestradiol-3-acetate-17-propionate. 250 mg. oestradiol-17-propionate (obtained by partial saponification of oestradiol-3:17-dipropionate) were dissolved in 1.5 ml. of pyridine and mixed with 0.5 ml. acetic anhydride. After standing at room temperature for a short time it was heated to 100° for 30 min. and worked up in the same way. Oestradiol-3-acetate-17-propionate was recrystallized several times from methanol-water and formed fine crystals with constant m.p. 112.7–113°. (Found: C, 75.10; H, 8.21 %. $C_{23}H_{30}O_4$ requires C, 74.55; H, 8.17 %.)

Oestradiol-3-acetate-17-benzoate. The acetylation of oestradiol-17-benzoate (obtained by partial saponification of oestradiol-3-*n*-butyrate-17-benzoate) in pyridine with acetic anhydride was carried out in the usual way. Although it was recrystallized several times and had constant m.p. 161–161.5°, the substance was not analytically pure.

Test methods

These substances were investigated both as regards dosage and method of testing by the oestrus and uterus growth tests exactly as described in the previous paper. Young uncastrated rats were again used for the uterus growth test. Since, however, the actions of the majority of the esters last more than 30 days and since the animals, on account of the onset of sexual maturity, are then no longer suitable for this test, supplementary experiments were made using castrated rats. The results obtained will be completed at a later date.

The points given in the curves represent the average values obtained on at least 3 animals. 1350 animals were used for these experiments.

The assay in the oestrus test

Free oestradiol. On the basis of numerous experiments carried out according to our method, the threshold value of oestradiol dissolved in sesame oil was found to be 0.4 γ for the rat. The same figure was also obtained by Schoeller *et al.* [1935]. It is about half that for oestrone (0.7 γ). In experiments made by other authors values varying from 0.07 to 0.6 γ were found according to the technique used.

Fig. 1 shows that even with relatively large doses (50 γ) the effect of oestradiol is only of short duration. In this respect it is entirely similar to oestrone. Dissolved in sesame oil, palm-kernel oil or glycerol a total dose of 50 γ oestradiol ($2 \times 25 \gamma$) causes 4 days' oestrus, but, dissolved in wool fat, 5 days.

Activators. Table II shows the influence of various activators on the duration of oestrus obtained with a total dose of 50γ oestradiol (in sesame oil) in the castrated rat.

Table II. *Effect of activators added to a dose of $2 \times 25\gamma$ oestradiol*

Activators	Duration of oestrus in days
—	4
100 mg. Palmitic acid	5-6
200 „ Palmitic acid	10 (7)*
200 „ Ricinoleic acid	4-5
200 „ Linoleic acid	4
200 „ Cetyl alcohol	8
200 „ Stearyl alcohol	24 (13)*

* The figures in brackets are for oestrone.

It will be seen that the activity of the true follicular hormone, oestradiol, is enhanced much more easily than that of the excretion product oestrone.

Di-esters

(a) *Aliphatic di-esters.* As will be seen from Table I and also from Fig. 1 the threshold values (S_7) determined on the rat for esters up to the dipropionate vary only slightly from that of free oestradiol, being $< 1\gamma$. With higher esters they rise quickly, reaching already with the di-*n*-decanoate 25γ which is almost the maximum, the dipalmitate having a value only 2γ more.

Fig. 1. The effect of oestradiol, oestradiol-3-benzoate and aliphatic di-esters of oestradiol on castrated rats in the oestrus test, using various doses.

It is clear from the curves of Fig. 1 that the longer the carbon chain of the acid used the greater the duration of oestrus obtained with the aliphatic di-esters. In general the duration of oestrus increases with the threshold value; with the lower di-esters the duration of effect increases more rapidly. Thus the duration of oestrus is 42 days with the dipropionate in a dose of 50γ whilst with the di-*n*-decanoate and the dipalmitate, the duration of effect amounts to almost 5 months.

In Fig. 2 the effects of the aliphatic oestradiol di-esters are compared with those of the corresponding oestrone esters as regards their threshold value (S_r) and duration of effect (T_{oe}). With the lowest esters of both series the threshold values are approximately the same; with the medium esters they are higher for oestradiol di-esters whilst for the highest esters the relationship is reversed. It must be observed that the number of carbon atoms of both acid radicals of the oestradiol di-esters is twice that of the corresponding oestrone esters. As regards duration of oestrus, the oestrone esters show a pronounced maximum; as we had assumed, the maximum occurs with the oestrone-*n*-heptanoate.¹ The oestradiol di-esters are far superior to the oestrone esters. With the former the steady increase in the duration of effect up to the di-decanoate is striking. A fall is only to be expected with still higher esters of oestradiol than those investigated.

Fig. 2. Comparison of the aliphatic oestradiol di-esters [—] and oestrone esters [----] as regards the threshold value (S_r) and duration of effect (T_{oe}) in the oestrus test.

Oestradiol dipropionate is of special interest as it exhibits with a low threshold value a relatively intense and prolonged effect which is dependent upon the dose. As a comparison it might be mentioned that the effect of oestradiol-3-benzoate, the only ester of oestradiol used clinically hitherto, does not quite attain that of the diacetate and is considerably below that of the dipropionate.

(b) *Aliphatic-aromatic esters.* This series is subdivisible into two according to whether the benzoyl radical is in the 3- or in the 17-position. Table I and Fig. 3 show clearly that the series of the 3-benzoyl esters has more favourable properties since the threshold value increases less quickly with the increasing length of the aliphatic acid radical than in the 17-benzoyl series. The 3-benzoyl series exhibits a more favourable action also as regards duration of effect. The 3-benzoate-17-*n*-butyrate and -17-*n*-valerate are of particular interest. As regards effect the former is similar to oestradiol di-*n*-octanoate but in contrast to this it crystallizes. The 3-benzoate-17-*n*-valerate is better than the dipalmitate which exhibits the most prolonged effect, in the aliphatic series, since it possesses the same duration of effect (50 γ : 140 days) with a smaller threshold value (17 γ against 27 γ).

¹ M.P. approx. 55°, B.P. 185–190° 0.0005 mm. (Found: C, 78.48; H, 8.96%. $C_{25}H_{34}O_3$ requires C, 78.46; H, 9.15 %.) The threshold value in the oestrus test (rat) is $S_r = 2.5 \gamma$, the duration of effect is 17 days (with a total dose of 50 γ). The output of activity in the uterus growth test is 202 cg.-days ($D_{max.} = 10.2 \text{ cg.}$; $t_{max.} = 4\text{th day}$; $T = 38 \text{ days}$; $c = 16.5$).

(c) *Aromatic di-esters.* Only the dibenzoate was tested. The threshold value is very high (125 γ). The duration of effect with a total dose of 200 γ , that is only 1.6 times the threshold dose, shows considerable variation; on the average it is about 30 days (5 animals).

Fig. 3. The effect of mixed aliphatic-aromatic di-esters and oestradiol-3-benzoate on castrated rats in the oestrus test, using various doses.

Uterus growth test

As in the oestrus test oestradiol given by subcutaneous injection to young rats also exhibits a relatively transitory effect in the uterus growth test even in a dose of 50 γ (see Fig. 4). Nevertheless, the output of activity calculated according to the method given previously [1938] is almost double that for oestrone (20 cg.-days against 12 cg.-days). By the addition of stearyl alcohol a

Fig. 4. The effect of oestradiol, with and without stearyl alcohol, and some lower aliphatic oestradiol di-esters and oestradiol-3-benzoate on the weight of the uterus of young rats after administration of a total dose of 50 γ of hormone given in equal parts on two consecutive days.

pronounced and rapid increase of effect is obtained (output of activity 151 cg.-days).

(a) *Aliphatic di-esters (young animals)*. In this test the aliphatic oestradiol di-esters also show extraordinarily intense and prolonged effects (see Figs. 4 and 5). Even the diacetate is, as regards the time course of its effect, similar to the 3-benzoate. With a total dose of 50 γ a duration of effect of more than 30 days is attained with the 3-acetate-17-propionate and dipropionate as well as with all

Fig. 5. The effects of some higher aliphatic di-esters of oestradiol and of oestradiol-3-benzoate on the weight of the uterus of young rats after administration of a total dose of 50 γ of hormone given in equal parts on two consecutive days.

di-esters with larger acid radicals. As has already been observed in the earlier tests with esters of oestrone, the total duration of effect on the uterus is more, in some cases considerably more, than that in the oestrus test. Higher esters such as the di-*n*-decanoate and dipalmitate doubtless exercise on the uterus an effect lasting about 6 months. The diacetate attains its maximum effect on the 6th day and the dipropionate on the 12th day; the maximum effect of the higher esters setting in still later. The increase of effect appears to be considerably delayed particularly with the dipalmitate.

(b) *Aliphatic-aromatic esters (young animals)*. As in the oestrus test the aliphatic-aromatic esters show also in the uterus growth test a markedly protracted action (see Fig. 6). Even on the 30th day the total activity of all esters investigated is still maintained. The weights of the uteri attained vary between 190 and 220 mg. The increase of effect is as a rule quicker with the 3-benzoyl-esters than with the 17-benzoyl-esters. With the 3-benzoate-17-*n*-valerate it is delayed to the same extent as with the dipalmitate.

(c) *Aromatic di-esters*. While no total oestrus is obtained with 50 γ oestradiol dibenzoate, the same dose produces a slight increase in weight of the uterus, the maximal weight of the uterus being about 100 mg.

(d) *Tests with di-esters on castrated animals*. To supplement the tests on young animals the time course of effect of each of two aliphatic and mixed aliphatic-aromatic esters was examined on castrated rats also after 30 days (see Fig. 7). The values obtained follow on those observed on young animals. It will be seen that with the di-esters exhibiting prolonged effects, such as the di-*n*-decanoate, their maximal effects are maintained for a long time or, as with the

3-benzoate-17-*n*-butyrate, even continue to increase slowly. The latter ester produces, e.g. in a total dose of 50 γ , an average weight of the uterus of 230 mg. on the 50th day. These experiments will be extended.

Fig. 6. The effect of mixed aliphatic-aromatic di-esters of oestradiol and of oestradiol-3-benzoate on the weight of the uterus of young rats after administration of a total dose of 50 γ hormone given in equal parts on two consecutive days.

Fig. 7. The effects of aliphatic and mixed aliphatic-aromatic di-esters of oestradiol on the weight of the uterus of young uncastrated rats and castrated rats after administration of a total dose of 50 γ hormone given in equal parts on two consecutive days.

(e) *Systematic arrangement of the results of the experiments.* So far as the values obtained allow some of the compounds studied have been characterized systematically (see Table III) as was done with the esters of oestrone in the previous paper. Again the letters signify:

A, output of activity, $D_{\max}$, the maximal increase in weight of the uterus, $t_{\max}$, the day of the onset of the maximal effect, T the total duration of effect, D_m the average increase of weight of the uterus, G_o the weight of the uterus at the commencement of the experiment, $d_{\max}$, the maximal increase in weight of the uterus in %, d_m the average % increase in weight of the uterus, and c the efficiency coefficient.

Table III

Substance	Absolute values					Relative values		
	A cg.-days	D _{max.} cg.	t _{max.}	T days	D _m cg.	Based on G _o = 5 cg.		Based on oestradiol = 1 c
						d _{max.} %	d _m %	
Oestradiol	20.3	5.0	4	9	2.3	100	46	1.0
Oestradiol + 200 mg. stearyl alcohol	150.6	15.0	4	27	5.6	300	112	7.4
Oestradiol diacetate	165.6	10.3	6	22	7.5	206	150	6.7
Oestradiol-3-acetate-17-propionate	278.8	11.0	10	33	8.5	220	169	13.7
Oestradiol dipropionate	567.2	15.0	12	60*	9.5	300	189	27.9
Oestradiol di- <i>n</i> -decanoate	1200.0*	—	—	80*	15.0	—	300	60.0
Oestradiol-3-benzoate	118.8	10.0	8	24	4.9	200	99	5.9

* Estimated.

Comparison with earlier experiments

Last year Parkes [1937] demonstrated the transitory effect of oestradiol in the plumage test on brown leghorns. According to his findings oestradiol diacetate was considerably better than oestrone acetate; oestradiol-3-benzoate-17-acetate proved to be the most effective, whilst oestradiol dibenzoate was effective only in very high doses. The high threshold value of the dibenzoate in the oestrus test (mouse) had also been observed by David *et al.* [1935]. Thus there is a satisfactory agreement between the findings of other authors and the results obtained on the rat by us with these compounds.

SUMMARY

1. Like free oestrone, free α -oestradiol exhibits only a transitory effect in the oestrus test on the rat even when given in high doses. Activators can considerably increase the duration of effect. In the uterus growth test oestradiol has a more intense effect than oestrone (output of activity 20 cg.-days against 12 cg.-days).

2. 10 aliphatic and 7 aliphatic-aromatic di-esters of α -oestradiol in addition to 1 aromatic di-ester were tested on the rat by subcutaneous injection (aromatic radical = benzoyl).

3. *Oestrus test.* In the aliphatic series the threshold value increases at first slowly up to the dipropionate and then more quickly up to the di-*n*-decanoate and dipalmitate. All di-esters exhibit very prolonged effects. The effect of the mixed aliphatic-aromatic di-esters is somewhat similar to that of the aliphatic di-esters with a similar number of carbon atoms. Nevertheless, the 3-benzoyl esters with an aliphatic acid radical in the 17-position are superior to their isomeric 17-benzoyl-di-esters.

4. *Uterus growth test.* In the uterus growth test using uncastrated young rats and also castrated rats all di-esters tested exhibit extremely intense and prolonged effects.

5. The oestradiol di-esters have on the whole much more intense and more prolonged effects than are attained even with the best oestrone esters as, e.g. oestrone-*n*-heptanoate.

REFERENCES

- David, de Jongh & Laqueur (1935). *Arch. int. Pharmacodyn.*, **51**, 137.
 Miescher & Scholz (1937). *Helv. chim. Acta*, **20**, 263, 1237.
 ——— & Tschopp (1938). *Biochem. J.* **32**, 141.
 Parkes (1937). *Biochem. J.* **31**, 579.
 Schoeller, Dohrn & Hohlweg (1935). *Klin. Wschr.* **14**, 826.