CLXIX. THE ACTIVATION OF FEMALE SEX HORMONES

III. MONO-ESTERS OF α -OESTRADIOL

By K. MIESCHER, C. SCHOLZ AND E. TSCHOPP

From the "Ciba" Research Laboratories

(Received 16 May 1938)

Two series of mono-esters are derived from α -oestradiol according to whether the acid radical is in the 3- or 17-position.

Up to the present only one mono-ester has been thoroughly investigated, viz. oestradiol-3-benzoate prepared by Schwenk & Hildebrandt [1933]. According to Kaufmann [1933; see also Schoeller *et al.* 1935] it has a protracted effect in comparison with the free follicular hormone. In the previous paper of this series [Miescher *et al.* 1938, 2] we showed that as regards duration of effect and output of activity many di-esters of oestradiol are much better than the 3-benzoate. The same applies also to the series of the 3- and 17-mono-esters. The investigation of the esters was made by determining their threshold value and duration of effect in the oestrus test as well as their action in the uterus growth test using rats in the same manner as described previously. Again the esters were injected subcutaneously in oily solution. 1275 animals were used for these experiments.

The mono-esters of α -oestradiol investigated by us are shown in Table I.

Table I

Oestradiol mono-esters	M.P.* ° C.	Oestrus test (rat)	
		Threshold value S_r rat oestradiol = 0.4 γ	Threshold value S_o based on oestradiol = 0.1 γ
3-Acetate	136.5-137.5	0.4	0.1
3-Propionate	125.0-126.0	0.4	0.1
3-n-Butyrate	98.0- 99.0	0.4	0.1
3-n-Valerate	Approx. 58.0- 60.0	0.4	0.1
3-n-Hexanoate	Approx. 46.0- 51.0	0.4	0.1
3-n-Octanoate	Approx. 48.0- 53.0	0.6	0.2
3-n-Decanoate	59.0- 60.0	2.0	0.5
3-Palmitate	70.0- 71.0	7.0	1.8
3-Stearate	78.0- 78.5	7.0	1.8
3-Benzoate	193.0-194.0	0.75	0.2
17-Acetate	215.0-216.5	0.5	0.1
17-Propionate	199.0-200.0	0.5	0.1
17-n-Butyrate	166.5-167.0	0.5	0.1
17-isoButyrate	183.0-183.5	0.5	0.1
17-n-Valerate	144.0-145.0	0.5	0.1
17-n-Hexanoate	128.5-129.0	0.7	0.2
17-n-Octanoate	117.5-118.0	0.8	0.2
17-n-Decanoate	112.0-112.5	3.5	0.9
17-Benzoate	93.0- 94.0	3.0	0.8
17-Methylcarbonate	216.5-218.0	0.6	0.2
17-Ethylcarbonate	171.0-172.0	0.6	0.2
3:17-Diethylcarbonate	137.5-138.0	1.5	0.4

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

Apart from the 3-benzoate only the two mono-acetates have been mentioned in the literature. The preparation of a series of mono-esters has already been reported by us elsewhere [Miescher & Scholz, 1937, 1, 2]. A description of some further mono-esters is appended.

Oestradiol-3-n-valerate. 630 mg. oestrone-*n*-valerate were dissolved in 95 ml. ethyl acetate (containing no alcohol) and mixed with 350 mg. platinum oxide (Adams). The whole was shaken at room temperature under a pressure of hydrogen of 4.3 atm. for 20 hr. After filtering off the platinum and evaporation of the solvent under diminished pressure the remaining oil was purified for analysis by two distillations at 0.0004 mm. (bath temperature 180–190°). (Found: C, 77.61; H, 9.01%. $C_{23}H_{32}O_3$ requires C, 77.47; H, 9.06%.) The oil can be crystallized from alcohol-water but the crystals contain solvent. m.p. about 58–60°.

Oestradiol-3-n-hexanoate. A solution of 428 mg. oestrone-*n*-hexanoate in 65 ml. alcohol-free ethyl acetate was mixed with 220 mg. platinum oxide and reduced as above. The working up was the same. After distilling 3 times at 0.0008 mm. (bath temperature 180–190°) a colourless oil was obtained which could be crystallized from alcohol-water; however, the crystals contain solvent. The m.p. is about 46–51°. (For the oil, found: C, 77.81; H, 9.20%. $C_{24}H_{34}O_3$ requires C, 77.78; H, 9.26%.)

Oestradiol-3-n-octanoate. In the same manner oestrone-*n*-octanoate, m.p. 69.8–70.2°, was reduced by means of platinum oxide in ethyl acetate and an oily residue obtained which was distilled 3 times at 0.0004 mm. (bath temperature 180°). The oil was dissolved in alcohol-water and after standing, crystals were formed, containing solvent. m.p. about 48–53°. (For the oil, found: C, 78.45; H, 9.46%. $C_{26}H_{38}O_3$ requires C, 78.33; H, 9.62%.)

Oestradiol-3-n-decanoate. The oestrone-*n*-decanoate was reduced in the manner described above. After filtering and evaporation of the ethyl acetate under diminished pressure an oil remained which after recrystallizing several times from methanol gave fine needles with constant m.p. 59–60°. (Found: C, 77.05; H, 9.97%. $C_{28}H_{40}O_3$, 0.5 H₂O requires C, 77.18; H, 9.95%.)

Oestradiol-17-n-hexanoate. 960 mg. oestradiol-di-*n*-hexanoate (an oil) were mixed with 60 ml. of 0.5% K_2CO_3 in 95% methyl alcohol and stirred for about 3 hr. at room temperature during which time solution occurred. The solution was then slightly acidified and by slowly adding water crystals were precipitated. After filtering off and thoroughly washing first with water and then with diluted Na_2CO_3 and again with water, almost pure oestradiol-17-*n*-hexanoate was obtained and recrystallized several times from methanol-water. m.p. 128.5–129°. (Found: C, 77.67; H, 9.29%. $C_{24}H_{34}O_3$ requires C, 77.78; H, 9.26%.)

Oestradiol-17-n-octanoate. Partial saponification of oestradiol-di-*n*-octanoate (an oil) with 0.5% K_2CO_3 in 95% methyl alcohol was carried out as above. The working up was the same. After purifying by recrystallization several times from aqueous methanol the crystals had m.p. 117.5–118°. (Found: C, 78.37; H, 9.85%. $C_{26}H_{38}O_3$ requires C, 78.33; H, 9.62%.)

I. Assay in the oestrus test

3-Mono-esters. As will be seen from Table I the threshold value in the oestrus test, determined on the rat, is the same from the 3-acetate up to the 3-hexanoate, and is the same as that of free oestradiol ($S_r = 0.4\gamma$). From the 3-octanoate it increases but does not exceed 7γ even with the palmitate and stearate. As regards duration of oestrus (see Fig. 1) with doses of 50γ only a slight increase of the duration of effect is to be seen up to the hexanoate (from 4 to 8 days). With the 3-octanoate the effect increases suddenly up to 52 days and attains with the 3-stearate a maximum of 80 days¹. On the other hand, the duration of effect with similar doses of the 3-benzoate is only 15 days. With the lower esters up to the 3-octanoate, oestrus appears on the 4th day, that is, one day later than with oestradiol. With the higher esters oestrus appears on the 5th day.

17-Mono-esters. In comparison with the 3-mono-esters the threshold value of this series is slightly increased. The most pronounced difference is observed with

¹ According to a private communication from Prof. Schoeller, Berlin, the 3-palmitate in a previous experiment on a monkey also showed a long-lasting effect.

the 17-benzoate (3γ) compared with the 3-benzoate (0.75γ). As regards the duration of oestrus (Fig. 2) the 17-mono-esters exceed the corresponding 3-mono-esters considerably. Using 50γ the 17-acetate and 17-propionate show the same duration of effect as the 3-benzoate (15 days). With the higher esters it increases

Fig. 1. The effects of aliphatic 3-mono-esters of oestradiol in comparison with oestradiol and its 3-benzoate on castrated rats in the oestrus test, using various doses.

rapidly. The 17-octanoate is of particular interest as, especially with comparatively small doses, it exhibits the most intense action of all esters hitherto investigated. With 5γ the oestrus lasts 30 days, with 20γ 60 days and with 50γ 86 days. With the higher esters (decanoate) the effect appears to diminish. The duration of effect of 17-benzoate (60 days) is 4 times that of the 3-benzoate (15 days). With the 17-mono-esters onset of oestrus is rather more rapid than with the 3-mono-esters. All the 17-mono-esters investigated show their full effect on the 4th day.

Fig. 2. The effects of 17-mono-esters of oestradiol in comparison with oestradiol and its 3-benzoate on castrated rats in the oestrus test, using various doses.

In Fig. 3 the threshold values (S_r) and duration of oestrus (T_{oe}) of the 3- and 17-mono-esters (with doses of 50γ) are given for comparison. The superiority of the 17-esters over the corresponding 3-esters as regards duration of oestrus is clear. A comparison of this figure with Fig. 2 in the previous paper [1938, 2] is

very instructive. It will be seen that with doses of 50γ none of the 3- and 17-mono-esters studied is equal to the most active di-esters as regards duration of effect.

In addition to these 17-mono-esters we examined also two 17-monocarboxylic esters as well as one 3:17-di-ester of oestradiol using a dose of 50γ . The duration of oestrus is with the 17-methylcarbonate 14 days, with the 17-ethylcarbonate 25 days and with the diethylcarbonate of oestradiol 40 days.

Fig. 3. Comparison of the aliphatic oestradiol 3-mono-esters (—) and 17-mono-esters (----) as regards the threshold value (S_r) and duration of effect (T_{oe}) in the oestrus test.

II. Uterus growth test

3-Mono-esters. As in the oestrus test the lower aliphatic 3-mono-esters show also in the uterus growth test (Fig. 4) first of all only a slight increase of effect

Fig. 4. The effects of aliphatic 3-mono-esters of oestradiol in comparison with oestradiol and its 3-benzoate on the weight of the uterus of young rats after subcutaneous injection of a total dose of 50γ of hormone given in equal parts on two consecutive days.

with the increasing length of the acid radical. On the other hand, the 3-octanoate, and particularly the higher esters, exhibit more pronounced effects. As regards effect the 3-benzoate is between the 3-hexanoate and 3-octanoate. The lower esters up to the 3-hexanoate attain their maximum effect on the 4th day. With the 3-octanoate it is delayed to the 20th day, the maximum effect of the higher esters appearing still later.

17-Mono-esters. The intensive action of the esters substituted in the 17-position is very obvious (Fig. 5). Already with the 17-propionate the intensity of effect obtained with the 3-benzoate is reached, yet as regards duration the former is superior (more than 30 days against 20 days). From the 17-*n*-valerate onwards all higher esters show particularly pronounced effects; even after 30 days the action is still maintained. In comparison with the 3-mono-esters the maximum effect of the corresponding lower 17-mono-esters is delayed. Generally it may be said that the higher the activity of the ester the later the maximum is reached.

Fig. 5. The effects of 17-mono-esters of oestradiol in comparison with oestradiol and its 3-benzoate on the weight of the uterus of young rats after subcutaneous injection of a total dose of 50 γ of hormone given in equal parts on two consecutive days.

The already mentioned carbonates were examined also as regards their effects in the uterus growth test. The 17-monoethylcarbonate with an output of activity of 204 cg.-days was twice as active as the 17-monomethylcarbonate (approx. 100 cg.-days). The diethylcarbonate of oestradiol shows an action similar to that of the oestradiol-17-*isobutyrate*.

III. Comparison of the effects on the uteri of castrated and uncastrated animals

Up to the present our results have been mainly obtained with young uncastrated rats. Experiments with oestradiol-17-octanoate and -dipropionate on a great number of castrated rats demonstrate that during the comparable interval of the first 30 days the corresponding values obtained agree in an astonishing way (see Fig. 6), especially considering the different weights of the

two kinds of animals. However, it should be taken into account that the average weight of the uteri of the castrated untreated rats which we used was approximately the same as that of the young rats. In any case the use of young uncastrated rats for the testing work during the first 30 days is quite justified.

Fig. 6. Comparison of the effects of oestradiol-dipropionate and oestradiol-17-octanoate on the weight of the uteri of young uncastrated rats (---) and of castrated rats (—) after subcutaneous injection of a total dose of 50γ hormone given in equal parts on two consecutive days.

IV. Characterization of the effect of the investigated compounds

As in the previous publications the effects of the compounds dealt with in this paper have been characterized systematically, in so far as complete data are available, according to their output of activity, A , onset of the maximal effect, T , etc. With all higher esters the output of activity is above 300 cg.-days and attains quite considerable values.

Table II

Substance	Absolute values					Relative values		
	A cg.-days	$D_{\max}$ cg.	$t_{\max}$ days	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
-3-acetate	22.6	4.0	4	11	2.0	80	40	1.1
-3-propionate	30.0	5.5	4	12	2.5	110	50	1.5
-3- <i>n</i> -butyrate	37.2	6.0	4	13*	2.9	120	58	1.8
-3- <i>n</i> -valerate	46.0	8.0	4	13	3.5	160	70	2.3
-3- <i>n</i> -hexanoate	59.6	9.0	4	14*	4.2	180	84	2.9
-3-benzoate	118.8	10.0	8	24*	4.9	200	99	5.9
-17-acetate	86.0	7.5	3	22	3.9	150	78	4.2
-17-propionate	194.0	10.0	8	34*	5.7	200	114	9.6
-17- <i>n</i> -butyrate	327.0	12.0	15	46*	7.1	240	142	16.1
-17- <i>n</i> -octanoate	1100.0	16.0	30	100*	11.0	320	220	54.0
-17-methylcarbonate	100.0	8.0	7	24*	4.2	160	84	4.9
-17-ethylcarbonate	204.0	9.0	7	37*	5.5	180	110	10.1

* Estimated.

V. Histological observations after administration of oestrone and oestradiol esters

It is known that the injection of follicular hormone into female rodents causes characteristic modifications in the structure of the mucous membrane of the uterus and vagina.

The modification produced by esters of oestrone and oestradiol depends on the dose given and on the intensity and duration of effect of the derivatives concerned. After giving rats 50γ (administered subcutaneously in two equal doses on

consecutive days) of one of the esters investigated by us, the histological picture of the uteri shows a pronounced hypertrophy of all tissue layers so long as the typical cornified non-nucleated epithelial cells are present in the vaginal smear. Macroscopic examination shows that this organ is enlarged considerably and during the first days of the experiment is filled with liquid and mucus. With the shorter-acting esters the liquid diminishes from about the 6th to the 15th day and from the 15th to the 20th day in the case of the longer-acting esters.

When the effect is mild, the epithelium of the mucous membrane consists first of all of high cellular elements in a single layer. With esters exhibiting a more intense and more prolonged effect other modifications occur which are to be seen particularly in the epithelium. Whereas in the musculature and the mucous membrane a large number of eosinophil leucocytes appear, characteristic changes are perceptible on the epithelium. In the place where single-layered cylindrical epithelium was to be expected, with esters having a prolonged effect several strata of partly keratinized cells of very high cylindrical epithelium ("vertical keratinization" according to Freud [1937]) are found. The multiplicity of layers can be very pronounced at certain places yet the cylindrical epithelial character always remains. An infiltrative growth with destruction of the limiting membrane or deep proliferation of solid epithelial cones, as described by Selye *et al.* [1935] after administration of high doses of follicular hormone was not observed in our experiments. When the effect of the hormone ceases then the cornified non-nucleated cells in the vaginal smear disappear, the uterus atrophies and instead of the cornified epithelial cells nucleated epithelial cells appear; these are finally replaced by normal cylindrical epithelial cells.

Only in isolated cases and with esters of oestradiol having a very prolonged effect, were we able to observe in castrated rats a change of the single-layered cylindrical epithelium of the mucosa into a multiple-layered pavement epithelium as described by Selye *et al.* [1935], Pierson [1935], Grumbrecht [1935], Korenchevsky [1937], Szarka & Rechnitz [1938], Dessau [1938] etc.

We must mention that in our rats, even when using high doses of esters of oestradiol having very prolonged action, malignant tumours were never observed.

Note. After completing this work we became aware of a publication by A. Fischer [1938] entitled: "Ueber die biologische Wirkung der Follikelhormon-Derivate." Probably following on our own publications [Miescher, 1937; Miescher *et al.* 1937, 1, 2, 3; 1938, 1, 2], this author also investigated esters of oestrone and oestradiol, particularly oestradiol-17-mono-esters and determined, as we did, besides the threshold value in the oestrus test also the duration of the oestrus and the effect on the uterus. The oestrus threshold values ascertained on rats agree with those communicated by us. To determine the duration of oestrus, Fischer gave a single dose of 1 γ to castrated mice and to ascertain the effect on the uterus he gave within 4 days 4 injections of 1.7 γ each to young rats and weighed the dried uterus on the 5th day. Fischer did not obtain satisfactory results with the uterus test and therefore he recommends the seminal vesicle test.

As is shown from the comparison with our results the experiments made by Fischer are to be considered as inadequate for the judgment of the effect of sex hormone compounds. The efficacy of a substance cannot be judged on the results of tests with small doses alone. A proper conclusion regarding the total activity of such a compound can only be made when higher doses are also taken into consideration. In the uterus growth test (and moreover also in the seminal vesicle test) the time course of the swelling and the subsidence of the swelling of the organ after injection must be considered. When this test is carefully carried out there is, contrary to the opinion of Fischer, a certain parallelism between the duration of the effect in the oestrus test and in the uterus growth test.

SUMMARY

1. Nine aliphatic 3-mono-esters, ten aliphatic 17-mono-esters and the 17-monobenzoate of α -oestradiol were tested by subcutaneous injection into the rat by the oestrus test and uterus growth test and the results compared with those obtained with oestradiol-3-benzoate.

2. *Oestrus test.* In both series of esters containing a fatty acid radical the threshold values are, up to the octanoates, comparatively low and are similar to that of free α -oestradiol (0.4 γ). From the octanoates they increase rapidly but do not exceed 7 γ for the esters investigated. The threshold values of the 17-mono-esters are generally somewhat higher than those of the corresponding 3-mono-esters. A prolonged effect is obtained particularly with the higher esters. In this respect all the 17-esters investigated are much superior to the corresponding 3-esters; the 17-octanoate exhibiting the most intense and prolonged effect even with small doses.

3. *Uterus growth test.* In this test also 17-mono-esters, particularly the lower esters, prove to be superior to the corresponding 3-mono-esters. The results of experiments with oestradiol dipropionate and the 17-mono-octanoate on uncastrated young and on castrated adult rats agreed in an astonishing way.

4. *Histological observations.* The single-layered cylindrical epithelium changes under the prolonged influence of hormone into several strata of partly keratinized cells of very high cylindrical epithelium ("vertical keratinization"). An infiltrative growth with destruction of the limiting membrane or deep proliferation of solid epithelial cones did not occur with our method of testing. Malignant tumours were not observed. When the effect of the hormone ceased a return to normal followed.

REFERENCES

- Dessau (1938). *Arch. int. Pharmacodyn.* **58**, 344.
Fischer (1938). *Arch. int. Pharmacodyn.* **58**, 332.
Freud (1937). *Acta brev. Neerl.* **7**, 179.
Grumbrecht (1935). *Arch. Gynaek.* **160**, 523.
Kaufmann (1933). *Klin. Wschr.* **12**, 1557.
Korenchevsky (1937). *J. Path. Bact.* **45**, 681.
Miescher (1937). *Schweiz. med. Wschr.* **67**, 1046.
—— & Scholz (1937, 1). *Helv. chim. Acta*, **20**, 263.
—— — (1937, 2). *Helv. chim. Acta*, **20**, 1237.
—— — & Tschopp (1937, 3). *Schweiz. med. Wschr.* **67**, 268.
—— — (1938, 1). *Biochem. J.* **32**, 141.
—— — (1938, 2). *Biochem. J.* **32**, 725.
Pierson (1935). *Z. Krebsforsch.* **41**, 103.
Schoeller, Dohrn & Hohlweg (1935). *Klin. Wschr.* **14**, 826.
Schwenk & Hildebrandt (1933). *Naturwissenschaften*, **21**, 177.
Selye, Thomson & Collip (1935). *Nature, Lond.*, **135**, 65.
Szarka & Rechnitz (1938). *Klin. Wschr.* **17**, 538.