STEROID LACTONES

I. THE OXIDATION OF ESTRONE ACETATE TO THE LACTONE ACETATE OF ESTROLIC ACID*

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The oxidation of a steroid ring ketone to a lactone according to the scheme

\[
\begin{align*}
\text{C}=\text{O} & \quad \longrightarrow \quad \text{C}=\text{O} \\
\end{align*}
\]

was first reported by Gardner and Godden (1), who obtained two lactones from the ammonium persulfate oxidation of coprostan-3-one. The production of one of these was later confirmed by Burekhardt and Reichstein (2), who obtained the lactone (I) by perbenzoic acid oxidation of coprostanone and reported the preparation of similar lactones from cholestan-3-one, methyl 3-keto-12(\(\alpha\))-acetoxycholanate, methyl 3-keto-11-cholenate, and methyl 3-ketochohanolate. These investigators reported further that under the conditions employed for the 3-keto compounds neither methyl 3(\(\alpha\))-acetoxy-12-ketochohanolate nor allopregnan-3(\(\alpha\))-ol-20-one acetate formed lactones. Recently, other lactones from 3-keto steroids have been prepared by Ruzicka, Prelog, and Meister (3) in connection with a study of the relationship between the structure and odor of certain androstane and etiocholane derivatives.

Westerfeld (4) has reported the oxidation of the 17-keto steroid, estrone, with alkaline hydrogen peroxide to form in low yield a sparingly soluble C\(_{18}\)-lactone melting at 335°, which was characterized by the formation of an acetate melting at 143.5–145° and a methyl ether melting at 166–168°. He formulated the reaction as involving the cleavage of Ring D, either between carbon atoms 16 and 17 or, more probably, 13 and 17, followed by the addition of the elements of hydrogen peroxide to give an intermediate dihydroxy acid (II), which lactonized on acidification of the reaction mixture to form III. Westerfeld, subsequently confirmed by Smith (5), established the estrogenic potency of the hydroxy lactone (III).

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STEROID LACTONES. I

![Steroid Lactones Diagram]

3b. \( R = H \)
IV. \( " = CH_3CO \)
V. \( " = CH_3CH_2CO \)
VI. \( " = C_2H_5CO \)
VII. \( " = CH_3 \)

VIII. \( R = H, R' = H \)
IX. \( " = H, " = Na \)
X. \( " = CH_3, R' = H \)
XI. \( " = H, R' = CH_3 \)
XII. \( " = CH_3, R' = CH_3 \)

(actually an alkaline solution of the corresponding dihydroxy acid or "open form") as being about one-fifteenth that of estrone. The latter investigator reported further that this material stimulated the secretion of
gonadotropic and adrenocorticotrophic hormones by the pituitaries of mature male rats to a much greater degree than could be accounted for alone by the estrogenic activity of the substance.

We have begun an investigation of the oxidation of a variety of keto steroids in order to prepare the lactones and their derivatives for physiological study. In this first report is described the oxidation of estrone acetate to form the lactone acetate (IV) of a dihydroxy acid (VIII) which for convenience has been given the name estrolic acid.

As a method for the preparation of estrolic acid derivatives, the oxidation procedure of Westerfeld was found to be unsuitable. Estrone acetate was therefore oxidized with a large excess of hydrogen peroxide in acetic acid solution. The crude crystalline lactone acetate, which was obtained in 57 to 63 per cent yield, was purified by alkaline hydrolysis, followed by carbonation of the aqueous alkaline solution to precipitate a small quantity of unaltered estrone. The purified hydroxy lactone (IIIb), which was obtained by subsequent acidification and heating, was then acetylated to produce pure IV; the corresponding propionate (V) and benzoate (VI) were also prepared.

The methyl ether (VII) of IIIb was prepared directly with methyl iodide and silver oxide or by dissolving (with saponification) the lactone in hot sodium hydroxide and treating the solution with dimethyl sulfate. From the alkaline saponification mixture of IIIb was also obtained the parent dihydroxy acid (VIII) by careful acidification with mineral acid in the cold, while the partial acidification of this alkaline solution with carbon dioxide afforded the crystalline sodium salt (IX) of the free phenol. The methyl ether (VII) was similarly saponified and the resulting chilled solution was acidified to produce the methyl ether acid (X). Treatment of the carboxylic acids VIII and X with ethereal diazomethane formed the methyl esters XI and XII, the first of which on treatment with benzoyl chloride in pyridine solution formed the benzoate of IIIb, instead of that of XI, together with a non-crystalline fraction. The latter, after alkaline hydrolysis, yielded a small crystalline fraction, m.p. 167-171°, which could not be successfully purified for analysis.

In an effort to characterize the third oxygen function of estrolic acid (VIII), several unsuccessful attempts were made to dehydrate its methyl ester; in all experiments the principal product was the hydroxy lactone (IIIb), with only traces, too small to manipulate, of other crystalline alteration products.

Pursuing this aim further, it seemed to us reasonable to suppose that the lactone itself, if it is of the δ-substituted δ-lactone type, might undergo Clemmensen reduction \(^1\) to give a mixture of stereoisomeric anhydrodihydro-

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\(^1\) Unpublished observations. See Martin, in Adams, R., Organic reactions, New York, 1, 162 (1942).
Steroid Lactones. I

Estrolic acids. The reduction of the lactone acetate (IV) with amalgamated zinc and hydrochloric acid in acetic acid was found to proceed smoothly, with the production of a mixture of crystalline acids, the resolution of which is being undertaken and will be described later.

The formulation of estrolic acid (VIII) as a 1,2-disubstituted 2-methyl-7-hydroxy-1,2,3,4,9,10,11,12-octahydrophenanthrene rests largely on its preparation from estrone and on the functional derivatives described above. The arrangement of the substituents at C1 and C2 as in IIa seems improbable on the grounds that this structure resembles that of the polyhydrophenanthrene carboxylic acids of the marrianolic acid type, in the esters of which the tertiary carbomethoxyl has repeatedly been observed to be more resistant to alkaline hydrolysis than the primary carbomethoxyl of an adjacent (C1) acetic or propionic ester group. The carbomethoxyl group in XI appears to be of the latter type, since it is easily hydrolyzed by warm, aqueous, methanolic potassium bicarbonate to form the hydroxy lactone (IIIb) in nearly quantitative yield.

It should be noted further than an acid of the partial structure IIa of the doisynolic acid type of compound would be expected to possess relatively high estrogenic activity. Actually, estrolic acid and its derivatives show less than 0.01 the activity of estrone.

Among the derivatives (IIIb, IV, and VII) of estrolic acid reported here which parallel those of III prepared by Westerfeld, there may exist chemical similarities, but their identity is seriously open to question on physiological grounds. Westerfeld's lactone (III) in the Allen-Doisy assay is over 6 times more active than IIIb administered in the same manner and, more strikingly still, it possesses pituitary-stimulating action, while estrolic acid and its derivatives actually inhibit the secretion of gonadotropic hormone by the pituitary glands of immature female rats.

Work in this laboratory is continuing on the oxidation of a variety of keto steroids and on the elucidation of the structure and configuration of the products obtained.

Experimental

Oxidation of Estrone with Alkaline Hydrogen Peroxide—Estrone (0.60 gm.) was oxidized at 30° in two experiments according to the method of

2 Numerous examples are to be found in the publications of Bachmann and co-workers on the synthesis of sex hormones (see (6) for the most recent paper of this series), and those of Miescher et al. ((7) Paper XVI) on the estrogenic carboxylic acids.

3 A full report of the physiological studies will be made in a separate paper.

4 This has been observed (private communication from Dr. O. W. Smith) in immature female rats as well as in mature males.

5 All melting points were determined in open capillaries with total immersion of
Westerfeld (4). The combined solid (0.36 gm.) obtained by acidification of the reaction mixtures was saponified with aqueous sodium hydroxide and the resulting cooled solution was saturated with carbon dioxide. This served to precipitate 0.13 gm. of unaltered estrone, which was removed by filtering the suspension through moist fullers' earth. The filtrate was acidified and warmed, and the precipitate (0.14 gm.) was collected. This "lactone" fraction was acetylated and the product was taken up in ether and washed successively with dilute hydrochloric acid, water, and sodium bicarbonate. The last removed 0.01 gm. of acidic material, which was discarded. The neutral fraction crystallized very slowly from aqueous methanol to give 0.09 gm. of solid melting at 124-135°. After further recrystallization, this melted at 127-130° and no pure substance IV could be obtained from it. The Allen-Doisy unit was found to be about 10 γ.

Oxidation of Estrone Acetate with Hydrogen Peroxide in Acetic Acid—To a solution containing 2.5 gm. of estrone acetate (m.p. 125-126°) in 20 ml. of glacial acetic acid were added 13.5 ml. of 30 per cent aqueous hydrogen peroxide. The solution was allowed to stand in the dark at 35° for 60 hours. The reaction mixture was then gradually diluted with water until the crystallization of the product was complete. In several such experiments, the crude product melted at 144-148° and was obtained in 57 to 63 per cent yield.

The aqueous mother liquor, which contained the alteration products of about 40 per cent of the estrone acetate used, was cooled, treated with sulfur dioxide, and concentrated at 40° under diminished pressure. The residue, part of which was sparingly soluble in water, provided no additional crystalline material.

In many orienting oxidation experiments the proportion of the reactants and the reaction temperature were varied within wide limits in establishing the optimum conditions described above.

Estrobolactone (IIIb)—The crude product (6.94 gm.) from several estrone acetate oxidations was suspended in 150 ml. of 20 per cent methanol containing 25 to 30 ml. of 10 per cent sodium hydroxide and heated on the steam bath until the solid dissolved (1 to 2 hours). The resulting solution was diluted with 75 ml. of water, cooled to 15°, and saturated with carbon dioxide (neutrality to phenolphthalein), the latter precipitating 0.32 gm.

the thermometer stem or with a thermometer graduated for partial stem immersion. Melting points marked "block" were determined in a preheated aluminum block and were taken as the temperature at which the compound actively melted during a period of 1 or 2 minutes. Samples for analysis were dried at 110° and 0.05 mm. over phosphorus pentoxide, except as otherwise noted. Microanalyses were performed by Dr. Robert T. Dillon and staff of the Analytical Division of G. D. Searle and Company, whose assistance in this work the author gratefully acknowledges.
of unaltered estrone. This was removed by suction filtration, with a small amount of fullers' earth as a filter aid, and the filtrate was strongly acidified with hydrochloric acid and warmed to 60–65° to promote the lactonization of the precipitated acid. After about 30 minutes, the suspension was cooled and the finely divided, crystalline lactone was collected. This material (5.7 gm.), melting at about 330° (block), served in most instances for the preparation of the functional derivatives described below. It is very sparingly soluble in most organic solvents and was purified by recrystallization from cyclohexanone or methyl cellosolve, from which it separated in the form of small prisms melting at 339° (block). Westerfeld (4) reported a melting point of 335–340° for his lactone. The sample for analysis was dried at 135°.

$C_{14}H_{22}O_3$. Calculated. C 75.49, H 7.74
Found. " 75.23, " 7.72
" 75.51, " 7.83

Esters of Estrololactone. (a) Acetate (IV)—The acetylation of 5.7 gm. of the precipitated IIIb was accomplished by heating it with 10 ml. of acetic anhydride in 15 ml. of pyridine for 2 hours on the steam bath. The product was decolorized by dissolving it in carbon tetrachloride and filtering the dried solution through a 2 × 8 cm. column of 80 mesh Alorco-A alumina. The colorless, crystalline residue from the eluate and washings crystallized from methanol in heavy, diamond-shaped crystals (5.51 gm.) melting at 149–150.5°. $[\alpha]_D^{24} = +42°$ (c = 0.277, chloroform). Westerfeld (4) reported 143.5–145° for the acetate of III.

$C_{18}H_{24}O_4$. Calculated. C 73.14, H 7.37
Found. " 73.47, " 7.48
" 73.28, " 7.49

(b) Propionate (V)—A suspension of 0.20 gm. of precipitated IIIb in 1 ml. of propionic anhydride and 1 ml. of pyridine was heated for 2 hours. The product crystallized from aqueous methanol in the form of needles melting at 146–148.5°.

$C_{20}H_{26}O_4$. Calculated. C 73.66, H 7.65
Found. " 73.72, " 7.70
" 73.93, " 7.80

(c) Benzoate (VI)—The benzoylation of 0.20 gm. of IIIb with 1 ml. of benzoyl chloride in 4 ml. of pyridine and recrystallization of the product from ethanol or acetone formed small needles which melted at 241–244°. The sample for analysis was dried at 135°.

$C_{24}H_{26}O_4$. Calculated. C 76.90, H 6.71
Found. " 77.19, " 6.78
" 76.91, " 6.74
Methyl Ether of Estroloactone (VII)—430 mg. of IIIb were saponified by refluxing the suspension in a solution of 0.17 gm. of potassium hydroxide in 20 ml. of n-propanol until the solid completely dissolved. After the alcohol was removed under diminished pressure, the residue in 10 ml. of water was shaken at 50–60° with several 0.2 ml. portions of dimethyl sulfate, the solution being maintained alkaline to phenolphthalein with 10 per cent potassium hydroxide. The separating solid began to turn yellow and gather into lumps after 1.0 ml. of dimethyl sulfate had been added. At this stage, the reaction mixture was acidified and the solid was saponified with aqueous methanolic potassium hydroxide. The hot, methanol-free solution was then strongly reacidified and the product was crystallized from aqueous methanol to give 0.38 gm. of small, flat needles melting at 167–172°. Repeated recrystallization served to raise the melting point to 172.5–174°. Westerfeld (4) reported 166–168° for the methyl ether of III.

C_{19}H_{24}O_3. Calculated. C 75.97, H 8.05
Found. " 75.94, " 8.00
" 76.06, " 7.92

The methyl ether was prepared from IIIb (0.22 gm.) with methyl iodide (5 ml.) containing silver oxide added in three 0.2 gm. portions during a refluxing period of about 30 hours. As the lactone slowly dissolved, a few lumps of Drierite were added to the boiling suspension. The crude crystalline product (0.15 gm.) melted at 167–171.5° and this melting point was not depressed by admixture with pure material prepared by the alkali-dimethyl sulfate procedure.

Estrolic Acid (VIII)—250 mg. of IV were saponified by heating with an excess of dilute aqueous sodium hydroxide and the cooled (15°) solution was slowly acidified with vigorous stirring to about pH 5 (faint darkening of Congo red) with hydrochloric acid. The solid acid was collected and recrystallized by dissolving it in warm acetone, removing by filtration a small amount of the sparingly soluble IIIb, and diluting the concentrated filtrate with water. The acid (0.15 gm.) separated in the form of small plates which melted with effervescence at 225° (block). If the melting point was determined in the usual way, the acid gradually lactonized without sintering and melted at about 330°. The sample for analysis was dried at 25°.

C_{18}H_{18}O_4. Calculated. C 71.03, H 7.95
Found. " 70.9, " 8.08
" 71.2, " 8.22

Methyl Estrolate (XI)—This ester was prepared by treating VIII with an excess of cold, ethereal diazomethane and crystallizing the product from aqueous methanol. The glistening needles which separated became
opaque on standing in a dry atmosphere and then melted at 95–97°. The sample for analysis was dried at 65° for 1 hour.

\[
\begin{align*}
\text{C}_{13}\text{H}_{20}\text{O}_4. & \quad \text{Calculated. C 71.67, H 8.23} \\
\text{C}_{13}\text{H}_{26}\text{O}_4 \cdot \text{CH}_3\text{OH.} & \quad \text{" " 68.54, " 8.63} \\
\text{Found.} & \quad \text{" 68.8, " 8.66} \\
& \quad \text{" 68.8, " 8.51}
\end{align*}
\]

When this was dried for 8 hours at 60–65°, the analytical values were C 71.2, H 8.34.

**Hydrolysis of XI**—A mixture of 0.10 gm. of pure XI and 0.07 gm. of potassium bicarbonate in 6 ml. of methanol and 4 ml. of water was refluxed on the steam bath for 10 minutes. Crystalline material began to separate during the first few minutes of boiling. The suspension was evaporated in a stream of air, the residue was shaken with 20 ml. of warm water, and the solid was collected. The product melted at 336–338° (block) and weighed 0.085 gm.

**Sodium Estrolate (IX)**—1.4 gm. of IV were saponified by heating with a mixture of 15 ml. of 10 per cent sodium hydroxide and 15 ml. of 10 per cent methanol, after which the solution was diluted with 50 ml. of water. This was cooled, saturated with carbon dioxide, and concentrated below 40° to yield the crude crystalline sodium salt (two crops from the concentrated sodium bicarbonate-sodium acetate solution). After purification by recrystallization from ethanol, there were obtained 1.35 gm. of fine needles (clusters) melting at 225° (block). The sample for analysis was dried at 25°.

\[
\text{C}_{18}\text{H}_{23}\text{O}_4\text{Na} \cdot 2\text{H}_2\text{O.} \quad \text{Calculated. C 59.65, H 7.51, Na 6.35} \\
\text{Found.} & \quad \text{" 59.5, " 7.92, " 6.21}
\]

Although the dihydrate appears to be stable, the salt dried at 100° is highly hygroscopic and quickly reverts to the dihydrate. In attempting to obtain the anhydrous material, Dr. Dillon reported that the carbon values approached those calculated for the hemihydrate, but were not increased further by prolonged heating in vacuo at 100°.

**Estrolic Acid Methyl Ether (X)**—The methyl ether lactone (VII) was saponified with aqueous methanolic sodium hydroxide and the methanol-free solution was carefully acidified, as in the preparation of VIII. The acid crystallized from aqueous acetone in the form of elongated hexagonal tablets melting at 135–136° with effervescence. The sample for analysis was dried at 25°.

\[
\text{C}_{13}\text{H}_{26}\text{O}_4. \quad \text{Calculated. C 71.67, H 8.23} \\
\text{Found.} & \quad \text{" 71.44, " 8.07} \\
& \quad \text{" 71.91, " 8.10}
\]
The methyl ether methyl ester (XII) was obtained by treating X with an excess of cold ethereal diazomethane and crystallizing the product from aqueous methanol. It formed glistening leaves which melted at 64.5–65.5° and, after drying at 65°, became opaque and then melted at 77.5–78.5°.

C₁₂H₂₃O₄. Calculated. C 72.26, H 8.49
Found (77.5 78.5°). " 72.2, " 8.56
" 72.5, " 8.64

Benzoylation of XI—In the attempted benzoylation of XI, a solution containing 0.56 gm. of the ester in 5 ml. of pyridine was heated with 2 ml. of benzoyl chloride for 2½ hours. After treatment with water, the reaction mixture was extracted with ether and the ethereal solution was washed successively with hydrochloric acid, water, sodium bicarbonate, and saturated sodium chloride solution. The residue from the ether solution was leached with warm methanolic acetone and the insoluble material (0.095 gm.), melting at 238–243°, was collected. By further recrystallization of the dissolved material from aqueous acetone and benzene-cyclohexane, 0.21 gm. of a faintly yellow, nodular solid was obtained, while the remainder of the product was non-crystalline. The combined solid crystallized readily from acetone in the form of very small needles (0.27 gm.) melting at 240–244°; mixed with the lactone benzoate (VI), this product melted at 241–244°.

The non-crystalline fraction was subjected to alkaline hydrolysis and the solution was acidified and warmed. The yellow gum which formed was leached with 80 per cent acetone, some insoluble solid was separated, and the solution was allowed to stand for several days. The finely divided solid, which was collected in several crops, was recrystallized from aqueous acetone to form a mixture of sparingly soluble powder and well formed blades. The latter (0.12 gm.), which were separated mechanically, melted at 167–171°. This fraction, after acetylation, failed to react with diazomethane and was recrystallized from ether-acetone, from which it separated as heavy blades, m.p. 168–172°. In an attempt to purify the “acetate” for analysis, the substance was so strongly adsorbed on the alumina that it could not be chromatographed. By leaching the alumina with hot 80 per cent acetic acid, about half (0.065 gm.) of the material was recovered and this was set aside for further study. When the benzoylation was conducted at 23° for 24 hours, the character of the products was the same.

Attempted Dehydration of XI—In several attempts to effect the dehydration of XI, the ester was treated (a) with acetic anhydride-pyridine and the non-crystalline product was then heated on the steam bath for 1 hour.
with phosphorus oxychloride in pyridine; (b) with 98 to 100 per cent formic acid for 3 hours on the steam bath or by refluxing for 1 hour; or (c) with iodine in refluxing toluene or xylene. In all instances the major product was the lactone IIIb or its esters.

The Clemmensen reduction of the lactone acetate (IV, 0.36 gm.) with amalgamated zinc in boiling acetic-hydrochloric acid produced 0.01 gm. of IIIb and 0.27 gm. of acidic material. The acids crystallized partially from aqueous acetone in small needles, melting in the range 205–225°. The resolution of this mixture by the chromatographic separation of the acetylated methyl esters will be described in a later paper.

SUMMARY

The oxidation of estrone acetate with hydrogen peroxide in acetic acid is reported to form, in 57 to 63 per cent crude yield, the lactone acetate of estrolic acid. The partial characterization of this acid is described. The suspected non-identity of certain estrolic acid derivatives with similar products previously reported by Westerfeld (4) is discussed.

BIBLIOGRAPHY

STEROID LACTONES: I. THE OXIDATION OF ESTRONE ACETATE TO THE LACTONE ACETATE OF ESTROLIC ACID
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