277. *Chemical Actions of Ionising Radiations in Solution. Part IX. Radiation Chemistry of Sterols. The Action of X-Rays on (+)-Œstrone-b in Aqueous Solution.*

By Max Keller and Joseph Weiss.

It is shown that the action of *X*-rays on (+)-œstrone-b in aqueous systems leads to formation of a lactone which is identical with that first prepared by Westerfeld (*J. Biol. Chem.*, 1942, **143**, 177). The mechanism of its formation by *X*-rays suggests that the lactone ring is formed by a CO_2H group involving $C_{(17)}$ and an OH group at $C_{(16)}$. Comparison of Westerfeld's lactone with the lactone prepared from œstrone acetate by Jacobsen (*J. Biol. Chem.*, 1947, **171**, 61, 81) has shown that in the pure state they are identical.

In recent investigations we studied the action of *X*-rays on cholesterol, 3β-hydroxypregn-5-en-20-one (Keller and Weiss, *Experientia*, 1950, **6**, 379; *J.*, 1950, 2709), and cholic acid (Keller and Weiss, *J.*, 1951, 25) in aqueous systems. In continuation of this work we now report their action on (+)-œstrone-b, which is of interest since female sex hormones are connected with the incidence of certain forms of malignant growth (cf. Fieser and Fieser, "Natural Products Related to Phenanthrene," New York, 1949, 3rd edn., pp. 330 *et seq.*).

Our first object was again to isolate and to characterise the products of irradiation and consequently we had to employ fairly large doses of *X*-rays (of the order of 10^6 r.). The irradiations were carried out in (i) alkaline and (ii) aqueous acetic acid solutions, with *X*-rays (200 kv.) in the manner described earlier.

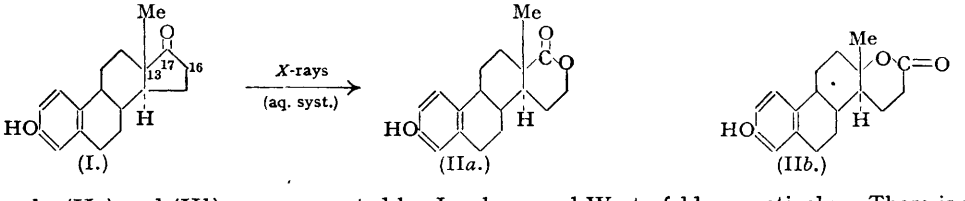
In N-sodium hydroxide 300 mg. of œstrone gave 212 mg. of starting material, 6 mg. of a crystalline compound (A), m. p. 310—312° (decomp.), and 14 mg. of an oil, which were separated chromatographically.

In 90% acetic acid chromatographic separation eventually yielded, from 300 mg. of œstrone, 133 mg. of starting material, 106 mg. of œstrone acetate, 4 mg. of a crystalline compound (B), m. p. 311—313° (decomp.), and 15 mg. of a brown oil.

Compounds (A) and (B), which showed no depression of the melting point when mixed, were in every respect identical. The relatively high melting point, low solubility in organic solvents, and analysis, which corresponded to the formula $C_{18}H_{22}O_3$, suggested that this substance was closely related to one of the lactones obtained by the oxidation of œstrone by hydrogen peroxide (Westerfeld, *J. Biol. Chem.*, 1942, **143**, 177) or by the oxidation of œstrone acetate by Jacobsen's method (*J. Biol. Chem.*, 1947, **171**, 61, 81). Although the melting points given for these lactones were somewhat higher (334—339°), Mather (quoted by Smith, *Endocrinology*, 1944, **35**, 146; *Proc. Soc. Exp. Biol. Med.*, 1945, **59**, 242) who repeated Westerfeld's procedure gives m. p. 309° for the lactone. As the relation between these two chemically prepared lactones was not clear and in order to compare them with the product obtained by the irradiation, we prepared these compounds and purified them carefully. We found that the lactone, prepared according to either Westerfeld or Jacobsen melted at 310—312° (decomp.) (Kofler block), and there was no depression of the melting point on mixing them or their acetates, and the optical rotations were ($[\alpha]_D^{21}$ +39·8° ± 4°, in pyridine). These lactones are thus identical. Identity with the compound obtained by the irradiation was proved by analysis and mixed melting point.

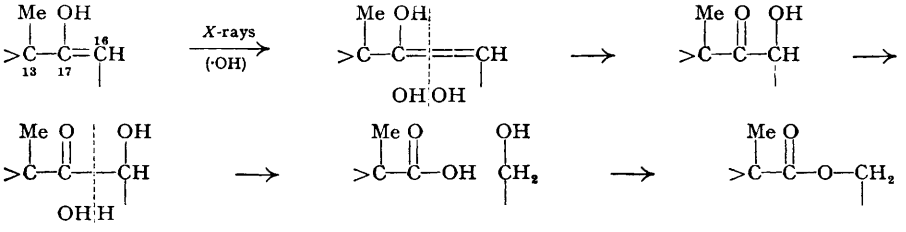
This lactone is interesting since Smith (*loc. cit.*) has shown that it stimulates the release of luteinizing hormone from the pituitaries of immature female rats, eliciting this response in smaller doses than does œstrone.

The formation of the œstrone lactone (II*a* or II*b*) from œstrone (I) corresponds to the net process :



Formulæ (II*a*) and (II*b*) were suggested by Jacobsen and Westerfeld respectively. There is no *definite* evidence at present to decide between these two structures but the character of the

CO_2H obtained by " hydrolysis " is evidence on the lactone structure in general. However, some conclusions can be drawn from the fact that the lactone is also formed by the action of *X*-rays. It is now fully established that ionising radiations produce hydrogen atoms and hydroxyl radicals in their action on water (Weiss, *Nature*, 1944, **153**, 748; *Trans. Faraday Soc.*, 1947, **43**, 314) and it is known from the previous work on steroids (Keller and Weiss, *loc. cit.*) that under the conditions in question double bonds are easily attacked by the hydroxyl radicals and can also be split under suitable conditions. In the five-membered ring of œstrone (I) a double bond can be only created between $C_{(16)}$ and $C_{(17)}$ (by enolisation of the carbonyl group at $C_{(17)}$, fission of which leads to structure (II*a*), as shown by the following scheme :



If one accepts this mechanism, structure (II*b*) is excluded as the tertiary character of $C_{(13)}$ does not allow the formation of a 13 : 17-double bond.

Experimental.

All m. p.s taken on the Kofler block (±2°).

Action of X-*Rays on* (+)-*Œstrone-b in Alkaline Solution.*—Œstrone (300 mg.; m. p. 258—260°), dissolved in N-sodium hydroxide (60 ml.), was irradiated with *X*-rays (200 kv.; *ca.* 3×10^6 r.) at 30—35°. The solution was then acidified (Congo-red), and the precipitate centrifuged, washed with water, dried in a desiccator, and treated with 50-ml. portions of ether, whereafter a colourless powder (P_1) remained (42 mg.). The ethereal extract was dried and the ether removed. The residue (240 mg.) was chromatographed through alumina (6 g.; standardised according to Brockmann). The column was prepared in benzene and the substance suspended in the latter. The column was eluted with 20-ml. portions of solvents. Benzene–ether (4 : 1 ⟶ 3 : 2) gave unchanged œstrone (200 mg.), m. p. 258—260° (from alcohol) not depressed by admixture with an authentic specimen. Chloroform gave a crystalline material (4 mg.), small prisms (1·2 mg.) (from alcohol), m. p. 311—312° (decomp.), darkening from 307°. The m. p. of this substance was not depressed on admixture with Westerfeld's lactone (*loc. cit.*) or with Jacobsen's lactone (*loc. cit.*). Further elution with chloroform–methanol (19 : 1) gave a brown oil (14 mg.). The powder (P_1) (42 mg.) was repeatedly recrystallised from alcohol, and the small prisms thus obtained (4·5 mg.) had m. p. 310—312° (decomp.), becoming brown from 307°, not depressed on admixture with Westerfeld's or Jacobsen's lactone. For analysis the compound was dried in a vacuum over phosphoric oxide at 130° (Found : C, 75·8; H, 7·9. Calc. for $C_{18}H_{22}O_3$: C, 75·5; H, 7·74%). The mother-liquor was chromatographed through alumina (1 g.), which was prepared as above. Benzene–ether (4 : 1) gave œstrone (12 mg.), m. p. 258—260° (from alcohol) not depressed on admixture with an authentic specimen. Elution with chloroform gave again a very small amount of the lactone, m. p. 310—312°, not depressed on admixture as above. Elution with chloroform–methanol (19 : 1) gave small amounts of an uncrystallisable brown oil.

Action of X-*Rays on* (+)-*Œstrone-b in Aqueous Acetic Acid.*—Œstrone (300 mg.; m. p. 258—260°) in 90% acetic acid (300 ml.) was irradiated with *X*-rays (*ca.* 3×10^6 r.) at 30—35°. The solvent was then removed in a vacuum at 35° and the oily residue extracted with ether. The ether-insoluble residue (9 mg.), after recrystallisation from alcohol, yielded small prisms (3·6 mg.), m. p. 310—312° (decomp.), not depressed on admixture with Westerfeld's or Jacobsen's lactone. The ethereal extract was washed with water, then dried (Na_2SO_4), and the ether removed. The oily residue (273 mg.) was chromatographed through alumina (8 g.), standardised according to Brockmann. Elution with light petroleum–benzene (1 : 1) in which the column was also prepared gave œstrone acetate (86 mg.), m. p. 125—126° (from methanol) not depressed on admixture with an authentic specimen. Elution with benzene gave crystals (~35 mg.) which were shown to be a mixture of œstrone acetate (~20 mg.) and œstrone (~10 mg.). Elution with benzene–ether gave unchanged œstrone (123 mg.), m. p. 258—260° (from alcohol) not depressed on admixture with an authentic specimen. Elution with chloroform gave small amounts of the lactone, m. p. 311—313° (from alcohol) not depressed on admixture with Westerfeld's lactone. Elution with chloroform–methanol (19 : 1) gave a non-crystallisable brown oil (15 mg.).

Preparation of Westerfeld's Lactone.—Œstrone (300 mg.; m. p. 258—260°) in N-sodium hydroxide (60 ml.) was treated with hydrogen peroxide (10%) (3·8 ml.), and the reaction allowed to proceed at room temperature and in the dark for 3 days. Subsequently the solution was acidified, and the precipitate collected, washed with water, and dried in a desiccator. The residue was extracted 8 times with 25-ml. portions of ether. The remaining residue, repeatedly recrystallised from alcohol, gave small prisms, m. p. 310—312° (decomp.), becoming brown from 307° (62 mg.); the mixed m. p. with Jacobsen's lactone was 310—313°. For analysis it was dried for 6 hours over phosphoric oxide in a vacuum at 130° (Found : C, 75·2; H, 7·8%). $[\alpha]_D^{21}$ was +39·8° ± 4° (*c*, 1·108 in pyridine).

The lactone (20 mg.) in pyridine (1 ml.) and acetic anhydride (0·5 ml.) was set aside for 48 hours at room temperature. The solvent was then evaporated in a vacuum, the residue dissolved in ether, washed until neutral, and dried (Na_2SO_4), and the ether removed. Recrystallisation from methanol gave diamond-shaped crystals (14 mg.), m. p. 147—149° not depressed on admixture with the lactone acetate prepared according to Jacobsen's method (*loc. cit.*).

Preparation of Jacobsen's Lactone.—To œstrone acetate (300 mg.; m. p. 125—126°) in acetic acid (3 ml.) was added hydrogen peroxide (32·5%; 2 ml.), and the solution was kept in the dark at 35° for 72 hours. The reaction mixture was then diluted with water until crystals appeared. After collection and drying, the crystals had m. p. 146—150° (205 mg.). Recrystallised from methanol, they had m. p. 148—150°, not depressed on admixture with Westerfeld's lactone acetate.

This acetate (140 mg.), dissolved in methanol (4 ml.), was treated with potassium hydroxide (1 ml.; 10%) and refluxed for 1½ hours. Subsequently 10 ml. of water were added and the methanol was removed in a vacuum. The precipitate formed on acidification (hydrochloric acid) was centrifuged, washed with water, and dried in a desiccator. Repeated crystallisation from alcohol gave small prisms (90 mg.), m. p. 311—313° (decomp.), not depressed on admixture with Westerfeld's lactone, and having $[\alpha]_D^{21}$ +39·4° ± 4° (*c*, 0·762 in pyridine).

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