

6. *Chemical Actions of Ionising Radiations in Solution. Part VII. Radiation Chemistry of Sterols. The Action of X-Rays on Cholic Acid in Aqueous Solution.*

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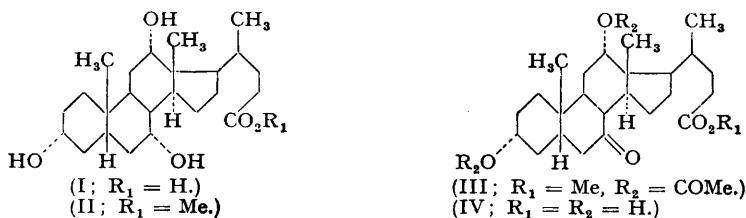
The action of X-rays on aqueous solutions of sodium cholate gives, as main product, $3\alpha:12\alpha$ -dihydro-7-ketocholanic acid. This substance is also formed in the first stages of the biological oxidation of cholic acid, thus showing again the parallelism between certain biological oxidations and those produced by penetrating radiations in aqueous systems.

IN previous publications (Parts I—IV, *J.*, 1949, 3241—3263; Parts V and VI, *J.*, 1950, 2704—2714) it has been shown that the action of ionising radiations, *e.g.*, X-rays, γ -rays, or neutrons (recoil protons), on dilute aqueous solutions is due to the formation of hydrogen atoms and hydroxyl radicals. In Part VI (*loc. cit.*) we studied the action of X-rays on cholesterol and on 3β -hydroxypregn-5-en-20-one in aqueous systems, and found that the hydroxyl radicals attack the double bond in the 5:6-position, leading to the formation of the corresponding (*trans*-)triols; in cholesterol, the CH_3 group at C_{17} is also attacked, being converted into a keto-group.

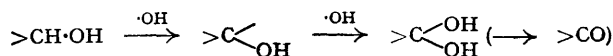
In view of the importance of steroid compounds in cell metabolism, we have now extended this study to other sterols, and report the action of X-rays on cholic acid (I). An approximately 0.5% aqueous solution of its sodium salt was irradiated with a dose of $\sim 1.8 \times 10^6$ r. units of X-rays (220 kv.) at about 35°. On elution chromatography after methylation of the crude product with diazomethane, about 80% of the starting material was recovered, and two oily fractions (*a*) and (*b*) were isolated. Fraction (*a*) ($\sim 4\%$) could not be crystallised. Fraction (*b*) ($\sim 7\%$) gave a crystalline product after acetylation which was shown

26 Chemical Actions of Ionising Radiations in Solution. Part VII.

to be methyl 3 : 12-diacetoxy-7-ketocholanate (III). Hydrolysis of this product gave 3 α : 12 α -dihydroxy-7-ketocholanic acid (IV), which is therefore the substance originally formed.



The production of this substance by these means is of considerable interest because it has been shown that this keto-acid is also formed as an intermediate in the biological oxidation of cholic acid by *Alcaligenes faecalis* (Hoehn, Schmidt, and Hughes, *J. Biol. Chem.*, 1944, 152, 59). This demonstrates—as already shown in Parts I—VI (*loc. cit.*)—that the action of X-rays on dilute aqueous solutions is similar to the processes in certain biological oxidations. This observation also sheds further light on the mechanism of the oxidative attack at the 7-position of the sterol structure. It has been suggested (Part VI, *loc. cit.*) that the oxidation of the CH₂ group in this position is a stepwise process proceeding through the intermediate formation of the CH·OH group. In the present case one starts with the CH·OH group and its transformation into the keto-group presumably corresponds to the two (final) stages :



EXPERIMENTAL.

(All m. p.s are uncorrected.)

1 G. of sodium cholate (I) (m. p. 195—196°) in 200 ml. of water was irradiated with a dose of $\sim 1.8 \times 10^6$ r. units of X-rays (220 kv.) at about 35°. The irradiated solution was acidified (Congo-red) with hydrochloric acid. The precipitated material was extracted with ether-chloroform, the extract washed with water and dried (Na₂SO₄), and the solvent removed in a vacuum. The residue was methylated with diazomethane in methanol-ether. The solvent was distilled off in a vacuum, the residue dissolved in ether, the extract washed till neutral and dried (Na₂SO₄), and the ether evaporated. The crude ester (about 1 g.) was chromatographed (30 g. of alumina, standardised according to Brockmann). The chromatographic column was prepared in a medium of light petroleum-benzene (1 : 1). Elution with benzene gave an oil (53 mg.), fraction (a), which did not crystallise even after acetylation and subsequent hydrolysis. Further elution with ether gave an oil (67 mg.), fraction (b). Elution with ether-chloroform mixtures gave methyl cholate (II) (810 mg.), m. p. 110—114°/156—158° (from methanol) (cf. Grand and Reichstein, *Helv. Chim. Acta*, 1945, 28, 344). Recrystallisation from absolute ether after drying in a vacuum gave a crystalline powder, m. p. 143—144°, not depressed on admixture with an authentic specimen.

Methyl 3 α : 12 α -Diacetoxy-7-ketocholanate (III).—Fraction (b) (67 mg.), which did not crystallise, was dissolved in 3 ml. of acetic anhydride and refluxed for 4 hours. The solvent was removed in a vacuum, and the residue dissolved in ether and washed till neutral. After drying, and evaporation of the solvent, the oily residue was crystallised from light petroleum and gave needles (47 mg.), m. p. 115—117° (Gallagher and Long, *J. Biol. Chem.*, 1943, 147, 131), not depressed in admixture with an authentic specimen of the diacetoxy-ester prepared by methylation and acetylation of the acid made according to Hoehn and Linsk (*J. Amer. Chem. Soc.*, 1945, 67, 312). For analysis the substance was dried in a high vacuum for 12 hours at 50° (Found : C, 68.9; H, 8.7. Calc. for C₂₈H₄₄O₇ : C, 69.0; H, 8.8%).

3 α : 12 α -Dihydroxy-7-ketocholanic Acid (IV).—The methyl ester (III) (40 mg.) was heated under reflux with a mixture of 1 ml. of methanol and 1 ml. of 2N-potassium hydroxide for 3 hours. The reaction mixture was diluted with water and acidified with hydrochloric acid. The precipitated crude acid, filtered off and washed with water, had m. p. 86—90° (dried in a desiccator). The acid (IV), when dried for several hours at 120° and crystallised from freshly distilled ethyl acetate, had m. p. 198—200° (6 mg.) (Gallagher and Long, *loc. cit.*; Haslewood, *Biochem. J.*, 1944, 38, 108), not depressed on admixture with an authentic specimen prepared according to Hoehn and Linsk (*loc. cit.*). For analysis the acid was dried for 12 hours in a high vacuum at 120° (Found : C, 70.5; H, 9.7. Calc. for C₂₄H₃₈O₅ : C, 70.9; H, 9.4%).

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