681. *The Action of Hydroxyl Radicals on Cholesterol and Some of its Esters.*

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When hydroxyl radicals, produced by Fenton's reagent, act on cholesterol or its acetate or hydrogen succinate in dilute aqueous acetic acid solution, the 5 : 6-double bond is hydroxylated and under suitable conditions the corresponding 7-ketone is also formed. These results are similar to those obtained previously with hydroxyl radicals produced by *X*-rays, on cholesterol in aqueous systems (Keller and Weiss, *J.*, 1950, 2709).

When a ferrous salt is added to a solution of hydrogen peroxide, hydroxyl radicals are produced :

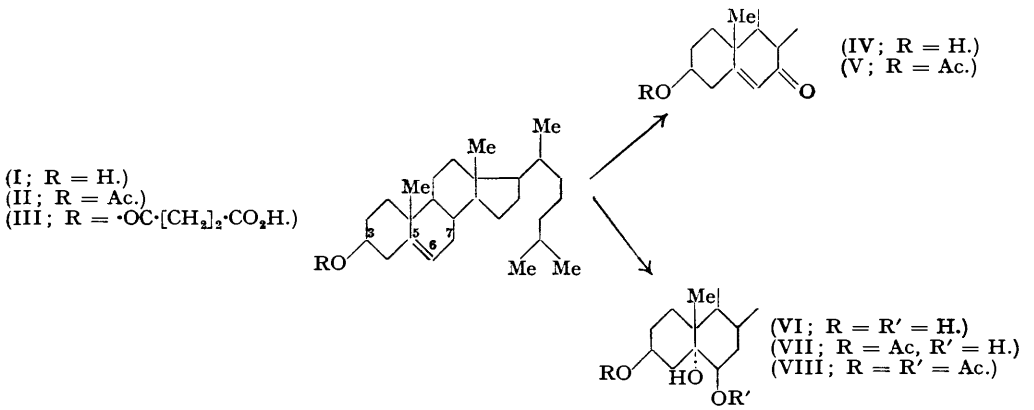
$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

(Haber and Weiss, *Proc. Roy. Soc.*, 1934, *A*, **147**, 332; Evans *et al.*, *Trans Faraday Soc.*, 1946, **42**, 155). The action of these radicals on a number of organic compounds has been studied recently (cf. Loebl, Stein, and Weiss, *J.*, 1949, 2074; 1950, 2704; Merz and Waters, *J.*, 1949, 2474; Stein and Weiss, *ibid.*, pp. 3245, 3254; see also Waters, " The Chemistry of Free

Action of hydrogen peroxide–ferrous salt reagent in dilute aqueous acetic acid at 35—40°.

Starting material.	Products.
Cholesterol (I)	Cholestane-3β : 5α : 6β-triol (VI) Cholestane-3β-acetoxy-5β : 6β-diol (VII)
Cholesteryl acetate (II)	Cholestane-3β-acetoxy-5α : 6β-diol (VII) 3β-Acetoxycholest-5-en-7-one (V)
	(After hydrolysis)
Cholesteryl hydrogen succinate (III)	Cholestane-3β : 5α : 6β-triol (VI) 3β-Hydroxycholest-5-en-7-one (IV)

Radicals," Oxford Univ. Press, 2nd edn., 1947). This is not only of preparative interest, but is also of general importance as relatively little is known about it and particularly as the radicals can be produced by the action of high-energy radiations (*X*-rays, etc.) on water (Weiss, *Nature*, 1944, **153**, 748; *Trans. Faraday Soc.*, 1947, **43**, 314). The action of *X*-rays on cholesterol and on 3β-hydroxypregn-5-en-20-one in aqueous systems has been investigated previously



(Keller and Weiss, *J.*, 1950, 2709). The effect of hydroxyl radicals, produced by Fenton's reagent, on cholesterol and its acetate and hydrogen succinate in aqueous acetic acid solution is summarised in the table. Clearly there is a general tendency for the hydroxyl radicals to be added to the isolated double bond in the 5 : 6-position, and under suitable conditions the 7-ketone was also isolated from the esters. It is very likely that the 7-ketone was also formed from cholesterol, but probably in quantities too small for isolation.

All the products isolated are similar to those obtained by the action of *X*-rays. In this case again the source of the hydroxyl radicals—chemical action or high-energy radiations—is without any appreciable qualitative effect. The present experimental material is not sufficient to afford a detailed explanation of the mechanism of these reactions (for a preliminary discussion see Keller and Weiss, *loc. cit.*).

Experimental.

(M. p.s are uncorrected.)

Action of Hydrogen Peroxide–Ferrous Reagent on Cholesterol.—To a solution of cholesterol (2 g.; purified by chromatography) in glacial acetic acid (500 ml.), hydrogen peroxide (50 ml.; 100 vols.) and a solution of ferrous sulphate (100 ml.; 5%) were added simultaneously under vigorous stirring at 40° during 1 hour. The resulting dark brown product was kept overnight at room temperature, evaporated to dryness at 35° in a vacuum, and extracted with ether. The ethereal extract was washed successively with dilute hydrochloric acid, sodium carbonate solution, and water till neutral, dried (Na_2SO_4), and evaporated, and the crude product (1·85 g.) chromatographed from benzene–light petroleum on alumina (60 g.), standardised according to Brockmann. Elution with 200-ml. lots of the solvents named gave the following fractions and products: (1) 1 : 1 Benzene–light petroleum, no product. (2—5) Benzene, cholesterol (380 mg.), m. p. 146—147° (from methanol). (6—12) 9 : 1 to 1 : 1 Benzene–ether, 3β-acetoxycholestane-5α : 6β-diol (480 mg.), m. p. and mixed m. p. 207—209° (from methanol) (Found : C, 75·4; H, 10·9. Calc. for $C_{29}H_{50}O_4$: C, 75·3; H, 10·9%). (13) 1 : 1 Benzene–ether, no product. (14—17) Ether, an oil (210 mg.). (18—22) 9 : 1 to 1 : 1 Ether–chloroform, no product. (23—25) Chloroform, an oil (80 mg.). (26, 27) 9 : 1 Chloroform–methanol, cholestane-3β : 5α : 6β-triol (600 mg.), m. p. and mixed m. p. 228—233° (from methanol) (Found : C, 76·6; H, 11·3. Calc. for $C_{27}H_{48}O_3$: C, 77·0; H, 11·5%). (28) 4 : 1 Chloroform–methanol and (29) methanol, no product.

The monoacetate with pyridine and acetic anhydride at room temperature gave 3β : 6β-diacetoxycholestan-5α-ol (VIII), m. p. 168—169° not depressed on admixture with authentic specimen (supplied by Dr. M. Davis, London) (Found : C, 73·7; H, 10·2. Calc. for $C_{31}H_{52}O_5$: C, 73·8; H, 10·4%).

Hydrolysis of the monoacetate with potassium carbonate in aqueous methanol gave the triol (VI), m. p. 228—233° after repeated crystallisations from methanol and ethyl acetate, not depressed on admixture with an authentic specimen.

The triol with pyridine–acetic anhydride gave the diacetate (VIII), m. p. 168—169° not depressed on admixture with an authentic specimen (Found : C, 73·6; H, 10·1%).

Action of Hydrogen Peroxide–Ferrous reagent on Cholesteryl Acetate.—This was carried out as described above for cholesterol. Elution with light petroleum–benzene (3 : 2) followed by crystallisation from methanol gave 3β-acetoxycholest-5-en-7-one (V) (60 mg.); m. p. and mixed m. p. 159—160°. Finally,

elution with benzene–ether (9 : 1 ⟶ 1 : 1) gave 3β-acetoxycholestane-5α : 6β-diol (VII) (250 mg.), m. p. and mixed m. p. 207—209° (Found : C, 75·2; H, 10·7. Calc. for $C_{29}H_{50}O_4$: C, 75·3; H, 10·9%). The monoacetate, as before, gave the diacetate, m. p. and mixed m. p. 168—169° (Found : C, 73·6; H, 10·2%), and hydrolysis gave the triol, m. p. and mixed m. p. 230—234°.

Action of Hydrogen Peroxide–Ferrous Salt on Cholesteryl Hydrogen Succinate.—A solution of cholesteryl hydrogen succinate (2 g.; m. p. 178—180°) was treated as described above. The crude product (~2 g.) was hydrolysed by refluxing it (2 hours) with potassium carbonate (4 g.) in boiling methanol containing 10% of water. After addition of more water the methanol was removed in a vacuum, the residue extracted with ether, the extract washed with water, dilute hydrochloric acid, and then again with water until neutral, and dried (Na_2SO_4), and the ether distilled off in a vacuum. The crude product (1·51 g.) was chromatographed from benzene–light petroleum on 60 g. of alumina. Elution with 200-ml. lots of the solvents named gave the following fractions and products: (1) 1 : 1 Benzene–light petroleum, no product. (2—6) Benzene, cholesterol (200 mg.), m. p. and mixed m. p. 146—147° (from methanol). (7—10) 9 : 1 to 3 : 2 Benzene–ether, no product. (11, 12) 1 : 1 Benzene–ether, 3β-hydroxycholest-5-en-7-one (15 mg.), m. p. and mixed m. p. 169—170° (from methanol) (Found : C, 80·4; H, 10·7. Calc. for $C_{27}H_{44}O_2$: C, 80·9; H, 11·0%). (13, 14) Ether, (15) 1 : 1 ether–chloroform, (16, 17) chloroform, no product. (18—20) 9 : 1 Chloroform–methanol, cholestane-3β : 5α : 6β-triol (1·1 g.), m. p. and mixed m. p. 230—235° (from methanol) (Found : C, 76·6; H, 11·2%).

Acetylation of the ketone, as above, gave the acetate (V), m. p. and mixed m. p. 159—160° (from methanol) (Found : C, 78·2; H, 10·2. Calc. for $C_{29}H_{46}O_3$: C, 78·7; H, 10·5%), and of the triol gave the diacetyl compound (VIII), m. p. 168—169° (Found : C, 73·4; H, 10·0%).

Our thanks are offered to the Medical Research Council for a maintenance grant for one of us (M. K.).

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