157 c.p.s. below absorption from protons in the water and two equal singlets for the allylic methyl groups at 168 and 193 c.p.s. above the solvent peak. The spectrum of the crude dimethyl ester, prepared with diazomethane in ether and examined in deuteriochloroform, was composed of two singlets for the allylic methyl groups at 95 (presumably *cis* to aryl) and 139 c.p.s. below the tetramethylsilane reference signal, two singlets, also from three protons each, for the carbomethoxyl groups at 217 and 230, a multiplet from three aryl protons at 425–455, and a multiplet from one aryl proton (presumably *ortho* to carbomethoxyl) at 475–487 c.p.s.

6α- and 6β-Hydroperoxy Derivatives of Δ⁴-Cholestenone[1]

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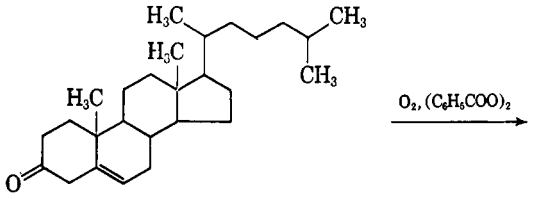
Some years ago, Fieser, Greene, Bischoff, Lopez, and Rapp[4] reported that 6β-hydroperoxy-Δ⁴-cholesten-3-one (**2**) injected in sesame oil in mice produced fibrosarcomas in 13 of 32 mice over a period of 12 months (17 survivors were negative at the time). The hydroperoxide was obtained by combination of Δ⁵-cholesten-3-one with molecular oxygen in hexane solution at 25°, but no preparative details were reported.



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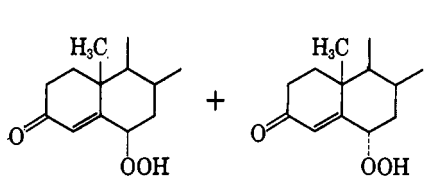

2 **3**

In view of recent interest in the oxidation of steroids[5] and of β,γ-unsaturated ketones,[6] and in the biological properties of the derived hydroperoxides, it may be of value to record a preparative procedure that affords both the 6α- and 6β-hydroperoxides of Δ⁴-cholestenone, separable by crystallization. Dibenzoyl peroxide was found to catalyze the autoxidation and cyclohexane proved to be a particularly favorable solvent. This is the method referred to by Fieser and Fieser[7] for the preparation of these hydroperoxides.

Experimental

Preparation and Separation of Hydroperoxides.—A mixture of 25 g. of Δ⁵-cholesten-3-one[8] and 580 ml. of cyclohexane was prepared at room temperature in a flask equipped with a reflux condenser and a tube for bubbling air through the liquid. While air bubbling was in progress the temperature was raised to 40–50°. After crystals of ketone had all dissolved 500 mg. of dibenzoyl peroxide was added, and air was bubbled through the solution at a temperature not exceeding 50° for 24–36 hr. At this point evaporation of an aliquot and titration by the method described below indicated the presence of 70–80% of hydroperoxide. The cyclohexane solution was cooled while air was still bubbling through it to aid in separation of the precipitate. This was collected by suction filtration, and concentration of the filtrate afforded further solid product. Crystallization of the total solid (22.5 g.) from ether (at room temperature, then at 0°) gave 6.4 g. of waxy flakes of the 6β-hydroperoxide. The filtrate was concentrated and let stand for several days, when two types of crystals were observed: waxy flakes of the β-hydroperoxide and more compact prismatic crystals of the 6α-hydroperoxide. The prisms (2.5 g.) were separated easily by dissolving away the more soluble flakes in ether. Further fractionation of the mother liquor material by the same method afforded a total of 9.8 g. of the 6β-isomer and 3.9 g. of the 6α-isomer; recrystallization from methanol gave 8.4 g. and 3.0 g. of the pure isomers.

Titration of Hydroperoxide.—The following is a modification of a standard method.[9] A flat-bottomed 300-ml. flask with a ground glass joint connected to a bubble trap containing water was charged with 1.5 g. of sodium bicarbonate and 20 ml. of acetic acid, and a weighed sample of hydroperoxide (up to 0.4 mmole) was dissolved in ethanol in a small vial which was placed upright within the reaction flask. Then 5 ml. of 40% potassium iodide solution was added to the outer liquid and the trap was connected. After about 2 min. carbon dioxide evolution had largely ceased and the flask was shaken to empty the vial and mix the contents. After 10–60 min. in the dark at room temperature the mixture was diluted with 30 ml. of distilled water and titrated with 0.01 *N* sodium thiosulfate solution (1 ml. = 2.08 mg. of cholestenone hydroperoxide).

6β-Hydroperoxy-Δ⁴-cholesten-3-one.—Constants found for fully purified material, m.p. 180–181°, $[\alpha]_D$ +27.2° (*c* 1.5, $CHCl_3$), λ^{EtOH} 235 mμ, agree with those previously reported.

6α-Hydroperoxy-Δ⁴-cholesten-3-one.—Repeated crystallization from methanol gave prisms, m.p. 150–151°, $[\alpha]_D$ 33.3° (*c* 1.5, $CHCl_3$), λ^{EtOH} 241 mμ.

Anal. Calcd. for $C_{27}H_{44}O_3$ (416.62): C, 77.83; H, 10.65. Found: C, 77.59; H, 10.80.

The possibility that the 6α-hydroperoxide arises by epimerization of the 6β-isomer during crystallization was tested by crystallizing 3.4 g. of the β-hydroperoxide from ether at different rates over a 3-week period. No prismatic crystals of the α-isomer could be identified.

Proof of Structures.—In a method adapted from a published one,[10] 50-mg. samples of the 6α- and 6β-hydroperoxide were each treated with 2 ml. of approximately 0.1 *N* lead tetraacetate in acetic acid. After 0.5 hr. at room temperature, addition of a solution of 1 g. of sodium chloride in 10 ml. of water caused precipitation of Δ⁴-cholestene-3,6-dione, which was extracted with ether and crystallized from methanol. The samples melted at 123–124° (λ^{EtOH} 251.5 mμ) and 121–122° and gave no depression when mixed with authentic Δ⁴-cholestene-3,6-dione,[11] m.p. 123–124°.

Reduction was accomplished by dissolving a 150-mg. sample of a 6-hydroperoxy-Δ⁴-cholesten-3-one in ethanol and treating with potassium iodide and acetic acid as in the titration procedure. After standing for 10–20 min., excess sodium thiosulfate solution was added together with enough water to precipitate the

(1) Dedicated to Professor Louis F. Fieser on the occasion of his 66th birthday for his distinguished contributions to teaching, research, and writing in organic chemistry.

(2) Address correspondence to the Stanford University School of Medicine.

(3) This work was carried out under the direction of Professor Louis F. Fieser in 1957–1958 while the author was on sabbatical leave of absence from Stanford University.

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product, which was extracted with ether and crystallized from methanol. The 6α-hydroperoxide gave 6α-hydroxy-Δ⁴-cholesten-3-one, m.p. 161.5–162.5°, $[\alpha]_D$ +79.1°, λ^{EtOH} 241.5 mμ. The 6β-isomer gave 6β-hydroxy-Δ⁴-cholesten-3-one, m.p. 188–189°, $[\alpha]_D$ +26.5°, λ^{EtOH} 237.5 mμ. The constants agree with those reported[8] and mixtures showed no melting point depression. Acetylation in pyridine gave 6α- and 6β-acetoxy-Δ⁴-cholesten-3-one of the following properties: m.p. 105–106°, λ^{EtOH} 238 mμ; m.p. 102.5–104°, Δ^{EtOH} 237 mμ.

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The Azine and *p*-Toluenesulfonylhydrazone of 12-Oxocholane*,[1]

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12-Oxocholane,[2] when refluxed with tosylhydrazine in ethanol solution, yields the expected tosylhydrazone I, but in addition, gives rise to a second nitrogen-containing compound II. The crystalline product II, m.p. 158.0–159.4°, has been assigned the structure of 12-oxocholane azine on the basis of elemental analyses, molecular weight,[3] and infrared evidence[4] of a –C=N– band (medium strong at 6.18 μ) and no N–H stretching bands, and the fact that it is quantitatively hydrolyzed to the original ketone.[4a] Synthesis of II by treatment of 12-oxocholane with hydrazine confirms the assignment of structure.

The formation of ketazine II in the reaction involving tosylhydrazine is of interest (1) because an azine has not been reported previously in a preparation of any tosylhydrazone despite considerable current concern[5,6] with the latter group of compounds, and (2) because tosylhydrazine undergoes pyrolytic breakdown to give diimide, an unstable intermediate capable of hydrogenating double bonds.[7]

Hydrazone I is not an intermediate in the reaction[8]; I, when refluxed under the original conditions, is unchanged. Instead, II results from reaction of the ketone with hydrazine freed when tosylhydrazine is refluxed in ethanol. Girard's reagents T and P undergo an analogous cleavage to form azines with ketones.[9]

Hydrazine is not present as a significant impurity in the tosylhydrazine used, as shown by a Pesez and Petit test.[10] However, when tosylhydrazine is refluxed in ethanol solution, after 24 hr. the test for hydrazine is distinctly positive, and addition of 12-oxocholane to the prerefluxed tosylhydrazine–ethanol solution produced the first indications of formation of azine within a few minutes (t.l.c.). In contrast, under the original conditions, in which ketone was mixed with tosylhydrazine and ethanol, and the mixture was brought to reflux, t.l.c. showed no formation of azine for at least 8 hr.; tosylhydrazone I, although detected initially at 90 min., increases in concentration very gradually, and even after 24 hr., a substantial proportion of 12-oxocholane is still present. Evidently the rate of reaction of the hindered ketone with tosylhydrazine in refluxing ethanol solution without added catalyst is so slow, that hydrazine, evolved also slowly from tosylhydrazine, can compete successfully for the ketone to form azine. With added hydrochloric acid, the reaction is sufficiently fast[11] that no azine is formed.

The finding that azine derived from hydrazine is a product of the reaction, whereas no hydrogenation products of either ketone or azine were detected, does not preclude the possibility that diimide is also a product of the breakdown of tosylhydrazine in refluxing ethanol. Van Tamelen[12] has shown that multiple bonds between heteroatoms are not reduced readily by diimide. In this connection, 12-oxocholane azine did not undergo catalytic hydrogenation in ethanol solution in the presence of platinum oxide.[13]

While Bamford and Stevens[5a] reported instances of conversion of tosylhydrazones to corresponding azines by treatment with bases, compound I, when refluxed with base, yielded only olefinic material[14]; no nitrogenous product was detected.

Experimental[15]

Reaction of 12-Oxocholane (12-Cholanone) with *p*-Toluenesulfonylhydrazine.—12-Oxocholane, m.p. 117.0–119.5°, $[\alpha]_D$ +104.9° (lit.[2] m.p. 115–117°, $[\alpha]_D$ +89.4°), 0.70 g., was heated at reflux for 48 hr. with 1.0 g. of tosylhydrazine (Aldrich Co.) in 40 ml. of absolute ethanol. On cooling, a crop of dense crystals, 0.21 g. (30%), m.p. 153–157°, separated from the solution. Recrystallization from ethanol yielded prismatic crystals, m.p. 158.0–159.5°, $[\alpha]_D$ +125.5°, mol. wt.[3] 684, $\lambda_{max}^{CS_2}$ 6.18 μ

* To Professor Louis F. Fieser.

(1) This investigation was supported in part by Grant CA-05011 from the National Cancer Institute, of the National Institutes of Health, U. S. Public Health Service.

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(11) However, 12-oxocholane reacts with tosylhydrazine much more slowly than does the 12-oxo steroidal sapogenin, hecogenin acetate; the latter was converted to the corresponding tosylhydrazone under similar acidic conditions in 30 min.[5b,c] Hecogenin acetate tosylhydrazone has been prepared also in ethanol without catalyst, but no corresponding azine was reported.[5b]

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(14) Studies of this product will be reported in the future.

(15) Microanalyses by Weiler and Strauss, Oxford. Melting points were determined on an electrical hot stage, uncorrected. Optical rotations were determined in 2% chloroform solution; infrared spectra on a Perkin-Elmer Infracord.