[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE RICE INSTITUTE]

Further Structural Evidence for the Reaction Product of Cholesterol-α-oxide and Methylmagnesium Iodide

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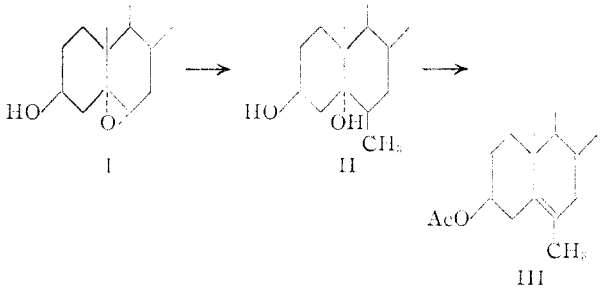
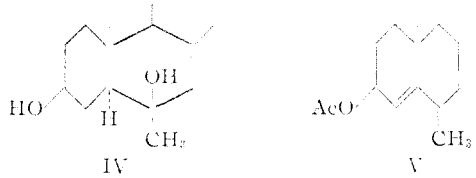
Cholesterol-α-oxide reacts with methylmagnesium iodide, but not with methyllithium nor with dimethylmagnesium, to yield a product that is shown by unambiguous methods to be 6β-methylcholestane-3β,5α-diol. Although no rearrangement occurs during the Grignard reaction, reaction of the oxide with magnesium bromide leads to the formation of Δ⁴-cholesten-6-one, cholestane-3β,5α,6β-triol and traces of 6-ketocholestanol. Oxidation of 6β-methylcholestane-3β,5α-diol gives 6β-methyl-5α-hydroxycholestane-3-one, which on dehydration with thionyl chloride and pyridine furnishes 6β-methyl-Δ⁴-cholesten-3-one, convertible into the 6α-epimer by the action of base. The present investigation confirms the finding of Fieser and Rigaudy that dehydration of the diol with acetic anhydride and sulfuric acid involves abnormal *cis*-elimination.

In 1939 Ushakov and Madaeva[1] observed that whereas cholesterol-α-oxide (I) does not react with methyllithium nor with dimethylmagnesium at temperatures of 80–100°, reaction with methylmagnesium iodide in refluxing benzene affords in 60% yield a diol, to which structure II was assigned.



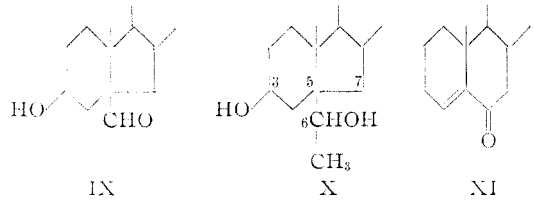
This substance furnishes a monoacetate and, with chromic acid, a difficultly soluble monoketone, which separates during the oxidation and which on treatment with hydrogen chloride in chloroform is converted into an α,β-unsaturated ketone, m.p. 126.5–127.5°, λ_{max} 240 mμ, log ε 4.25. Dehydration of the diol (II) with acetic anhydride containing a small amount of sulfuric acid gives an unsaturated product for which structure III, 6-methylcholesteryl acetate, was suggested. Subsequently Fieser and Fieser[2] pointed out that such a dehydration would involve *cis* elimination and proposed that, under the influence of magnesium iodide, cholesterol-α-oxide might first rearrange into 6-ketocholestanol, which on further reaction with the Grignard reagent would yield 6α-methylcholestane-3β,6β-diol (IV). Dehydration of the latter substance to III could then proceed by a concerted *trans* elimination mechanism.



Recently, however, Fieser and Rigaudy[3] showed that the diol IV derived from 6-ketocholestanol and methylmagnesium iodide differs from the diol of Ushakov and Madaeva, although dehydration of IV with acetic anhydride–sulfuric acid or of IV monoacetate with thionyl chloride and pyridine furnishes 6-methylcholesteryl acetate (III) identical with the product of the Russian investigators. On the other hand treatment of the monoacetate of II with thionyl chloride and pyridine gives an unsaturated derivative V as the major product. Only small amounts of 6-methylcholesteryl acetate are formed under these conditions. The structure of V was not rigorously established and was based primarily on structure II assumed for the starting diol.

Rearrangement of oxides under the influence of magnesium halides is well known.[4] Thus, although cyclohexene oxide and dimethylmagnesium yield *trans*-2-methylcyclohexanol, the reaction of cyclohexene oxide with methylmagnesium iodide furnishes cyclopentylmethylcarbinol.[5] Rearrangement of 1-methylcyclohexene oxide (VI) in the presence of magnesium bromide leads to the formation of acetylcyclopentane (VII) and of methylcyclopentylaldehyde (VIII).[6] If ring contraction were to occur in the case of cholesterol-α-oxide, inter-

mediate formation of an aldehyde IX, with subsequent conversion into a diol X, might be visualized. Acceptance of X as the structure of the



diol formed from cholesterol-α-oxide and methylmagnesium iodide would require the not unreasonable assumptions (a), that oxidation with chromic acid is interrupted at the monoketone stage owing to separation of the product from the reaction

(1) M. I. Ushakov and O. S. Madaeva, *J. Gen. Chem.* (*U.S.S.R.*), **9**, 436 (1939).

(2) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Ed., Reinhold Publishing Corp., New York, N. Y., 1950, p. 224.

(3) L. F. Fieser and J. Rigaudy, THIS JOURNAL, **73**, 4660 (1951).

(4) N. G. Gaylord and E. I. Becker, *Chem. Revs.*, **49**, 413 (1951).

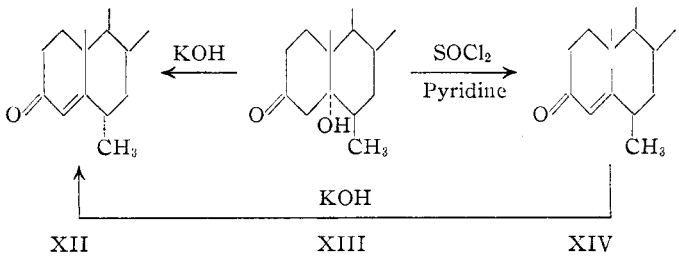
(5) P. D. Bartlett and C. M. Berry, THIS JOURNAL, **56**, 2683 (1934); M. Godchot and G. Cauquil, *Compt. rend.*, **186**, 375, 955 (1928); G. Vavon and V. M. Mitchovitch, *ibid.*, **186**, 702 (1928); P. Bedos, *ibid.*, **189**, 255 (1929).

(6) M. Tiffeneau and B. Tchoubar, *ibid.*, **207**, 918 (1938).

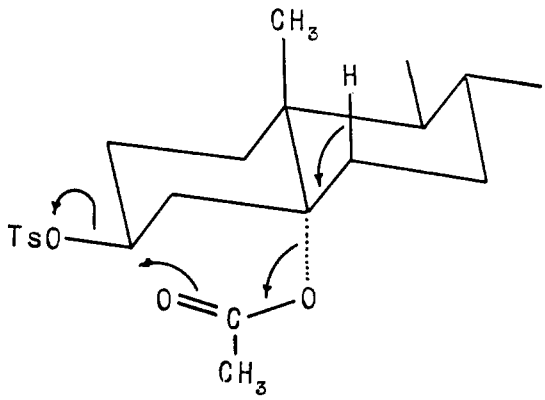
mixture and (b), that acetylation of the hydroxyl group attached to C.6 of formula X is strongly hindered. Apart from these restrictions the pinacolyl alcohol structure X provides a satisfactory formulation of the reaction product, and under acidic conditions its transformation into 6-methylcholesteryl acetate would be anticipated. Investigation of this possibility was therefore a matter of some interest.

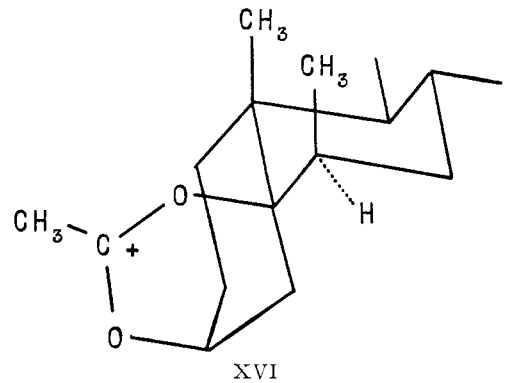
Cholesterol-α-oxide was treated with anhydrous magnesium bromide under conditions (refluxing benzene) employed in the Grignard reaction and yielded as the major product a substance with absorption characteristics (λ_{max} 241 mμ, ϵ_{max} 7,800) suggestive of an *s-cis*-α,β-unsaturated ketone.[7] The compound was identified as Δ⁴-cholesten-6-one (XI) by direct comparison with an authentic sample furnished by Dr. Hans Reich.[8] Smaller amounts of cholestane-3β,5α,6β-triol, apparently produced from traces of water or in the process of isolation, were also obtained in addition to traces of 6-ketocholestanol. In no case was an aldehyde of structure IX detected, and the possibility that the diol derived from cholesterol-α-oxide can have structure X was hence deemed unlikely. Structure II, however, is not uniquely determined by the previous work, since, with one exception, namely, conversion of the diol monoacetate into V, all reactions designed to establish the position of the second hydroxyl group were carried out in strongly acidic solution in which rearrangement cannot be excluded.

A 1,3-relationship of the two hydroxyl groups in the diol can, however, be demonstrated by certain reactions in which rearrangement is unlikely. Thus, Oppenauer oxidation of the diol,[9] or treatment of the hydroxyketone derived therefrom (chromic acid) with aluminum isopropoxide or with potassium hydroxide, furnishes an α,β-unsaturated ketone, m.p. 127–128.5°, λ_{max} 241 mμ, ϵ_{max} 16,650, identical in all respects with the unsaturated ketone of Ushakov and Madaeva, and with the Oppenauer oxidation product of 6-methylcholesterol. Since the structure of the latter compound is unequivocally established by Fieser's synthesis from 6-ketocholestanol,[3] the unsaturated ketone must possess either structure XII or XIV, whereas the hydroxyketone and parent diol must be 5α-hydroxy-6β-methyl derivatives (XIII and II, respectively) or the corresponding 6α-epimers. A 5α-hydroxy-6β-methyl structure is preferred for the diol on mechanistic grounds, and Fieser has advanced evidence in support of this contention based on molecular rotation data. A direct demonstration of the 6β-methyl configuration is provided by the following observations. Dehydration of the hydroxy ketone XIII with thionyl chloride and pyridine[10] furnishes an α,β-unsaturated ketone, m.p. 79–80°, λ_{max} 241 mμ, ϵ_{max} 14,800, epimeric with the previously mentioned unsaturated ketone of melting point 127° and convertible into it by reaction with base. Of the two compounds, XII and XIV, the latter possesses a polar methyl group at C.6 (β) which interacts strongly with the angular methyl group at C.10 and which can invert *via* the enol to the more stable equatorial configuration (6α). Formula XIV is hence assigned to the unstable ketone (m.p. 80°) from which structures XIII and II follow for the hydroxyketone and for the original diol, respectively.



The structure assigned to V is supported by the fact that Oppenauer oxidation of the free alcohol obtained from it by saponification furnishes 6α-methyl-Δ⁴-cholesten-3-one (XII) with inversion of configuration at C.6 as in the previous cases. The present investigation further confirms the observation of Fieser and Rigaudy that conversion of II into 6-methylcholesteryl acetate (III) cannot involve *trans* elimination to V followed by bond





(7) R. B. Turner and D. M. Voitle, THIS JOURNAL, **73**, 1403 (1951).

(8) H. Reich, F. E. Walker and R. W. Collins, *J. Org. Chem.*, **16**, 1753 (1951). The ultraviolet absorption of the semicarbazone of XI reported by these investigators is interesting in that it shows evidence of rotation from the planar configuration of the type observed in pulegone semicarbazone and in the semicarbazones of other *s-cis*-α,β-unsaturated ketones (see reference 7).

(9) *Cf.* L. Ruzicka and A. C. Muhr, *Helv. Chim. Acta*, **27**, 503 (1944).

(10) *Cf.* D. H. R. Barton and E. Miller, THIS JOURNAL, **72**, 370, 1066 (1950).

migration, for no recognizable products can be obtained from the reaction of V with acetic anhydride–sulfuric acid, whereas 6-methylcholesteryl acetate is stable to the action of these reagents.

There seems little likelihood that the transformation II → III can proceed by other than an E_1 mechanism (see reference 3). Whereas participation of an acetoxyl group by bridging between C.3 and C.5 has been observed in the solvolysis of 3β-*p*-toluenesulfonoxy-5α-acetoxycholestane (XV) to yield epicholesteryl acetate,[11] no evidence of participation of a 3β-acetoxyl group in reactions involving C.5 has been advanced. Indeed, although participation of the type formulated in XVI would provide a means of achieving a *trans* relationship between the C.6 hydrogen atom and the oxygen function at C.5, the geometry of the system (diequatorial) is not that which might be expected to facilitate elimination.[10,12]

Experimental[13]

Reaction of Cholesterol-α-Oxide (I) with Magnesium Bromide.—A solution of 2.70 g. of cholesterol-α-oxide in 50 ml. of dry benzene was added to a mixture of 3.80 g. of anhydrous magnesium bromide and 50 ml. of dry ether. The ether was removed by distillation, and the reaction mixture was then heated under reflux for 5 hours. After cooling, water was added and the benzene layer was washed successively with water, dilute sodium hydroxide solution, water and a saturated solution of sodium chloride. The solution was finally dried over anhydrous sodium sulfate and the solvent removed under reduced pressure.

Chromatography of the residual material furnished in the early eluates 500 mg. of Δ⁴-cholesten-6-one, m.p. 101–104°. Several recrystallizations from methanol gave a pure sample as long needles, m.p. 107–108°, λ_{max}^{alc} 241 mμ, ϵ_{max} 7,800. Admixture with an authentic sample[8] caused no depression of the melting point.

A second fraction, obtained by elution of the column with ether, gave crude material from which, after acetylation and crystallization from methanol, 45 mg. of a substance, m.p. 129–130°, was obtained that did not depress the melting point of 6-ketocholestanyl acetate.

Finally, elution with methanol followed by acetylation afforded 380 mg. of cholestane-3β,5α,6β-triol, m.p. 165–166°, identical in all respects with a known sample.

Oppenauer Oxidation of 6-Methylcholesterol.—A solution of 450 mg. of 6-methylcholesterol, prepared from 6-ketocholestanol as described by Fieser and Rigaudy,[3] and 500 mg. of aluminum isopropoxide in a mixture of 15 ml. of freshly distilled cyclohexanone and 20 ml. of dry benzene was heated under reflux for 14 hours. At the end of this time the reaction mixture was steam-distilled to remove the solvents and steam-volatile condensation products. The residue containing precipitated aluminum hydroxide was acidified with dilute hydrochloric acid and extracted with ether. The resulting ethereal solution was washed with water, dilute alkali, saturated sodium chloride solution, filtered through anhydrous sodium sulfate and concentrated to dryness. Crystallization from methanol furnished 350 mg. of 6α-methyl-Δ⁴-cholesten-3-one, m.p. 121–123°. Two recrystallizations from methanol gave a pure sample, m.p. 127–128.5°, $[\alpha]_D$ +60.5° (*c* 1.21, dioxane), λ_{max}^{alc} 241 mμ, ϵ_{max} 16,650.

Oppenauer Oxidation of 6β-Methylcholestane-3β,5α-diol (II).—A solution of 500 mg. of 6β-methylcholestane-3β,5α-diol[1] and 500 mg. of aluminum isopropoxide in a mixture of 10 ml. of cyclohexanone and 20 ml. of dry benzene was refluxed for 20 hours. The product was isolated as described in the preceding experiment, except that the aluminum hydroxide was removed by washing with alkali. The crude product after several recrystallizations from methanol gave 180 mg. of 6α-methyl-Δ⁴-cholesten-3-one, m.p. 125–127°, which did not depress the melting point of the sample prepared above.

Oppenauer Oxidation of 6β-Methyl-Δ⁴-cholesten-3β-ol (V).—A solution of 300 mg. of 6β-methyl-Δ⁴-cholesten-3β-ol[3] and 500 mg. of aluminum isopropoxide in a mixture of 10 ml. of cyclohexanone and 30 ml. of dry benzene was refluxed for 14 hours. The product was isolated as described above and after crystallization from methanol melted at 124–126°. A mixed melting point with a sample of XII showed no depression.

6β-Methyl-5α-hydroxycholestane-3-one (XIII).[1]—6β-Methylcholestane-3β,5α-diol (1.20 g.) was dissolved in 50 ml. of acetic acid with gentle warming. The solution was cooled to room temperature, and a solution of 350 mg. of CrO_3 in 25 ml. of 80% acetic acid was added slowly with stirring. After standing overnight at room temperature, the reaction mixture, containing large amounts of precipitated product, was diluted with ether and washed with water, dilute sodium hydroxide, saturated sodium chloride, filtered through anhydrous sodium sulfate and concentrated to dryness. The residue on crystallization from ethyl acetate gave 620 mg. of 6β-methyl-5α-hydroxycholestane-3-one, m.p. 227–228° (dec.),[14] $[\alpha]_D$ +20.5° (*c* 1.68, dioxane).

Reaction of 6β-Methyl-5α-hydroxycholestane-3-one (XIII) with Base.—A solution containing 200 mg. of XIII and 500 mg. of potassium hydroxide in 50 ml. of 90% aqueous methanol was refluxed in an atmosphere of nitrogen for 19 hours. After acidification with acetic acid, the reaction mixture was concentrated under reduced pressure, diluted with water and extracted with ether. The ethereal solution was washed with dilute sodium hydroxide, saturated sodium chloride, filtered through anhydrous sodium sulfate and concentrated to dryness. The crude product was purified by chromatography on alumina and, after crystallization from methanol, gave 6α-methyl-Δ⁴-cholesten-3-one (XII), m.p. and m.m.p. 124–126°.

Similar results were obtained when XIII was heated with a solution of aluminum isopropoxide in benzene.

Preparation of 6β-Methyl-Δ⁴-cholesten-3-one (XIV).—6β-Methyl-5α-hydroxycholestane-3-one (XIII) (550 mg.) was dissolved in 9 ml. of dry pyridine and cooled to 0° in an ice-bath. Redistilled thionyl chloride (0.3 ml.) was added dropwise to the cooled solution, and the reaction mixture allowed to stand at 0° for 10 minutes. Ice-water was then added, and the product taken into ether. The ethereal solution was washed successively with water, dilute hydrochloric acid, water, dilute sodium hydroxide, saturated sodium chloride, dried over anhydrous sodium sulfate and concentrated under reduced pressure at room temperature. The residue was crystallized from methanol and gave 420 mg. of material melting at 78–80°. Two recrystallizations from methanol gave the analytical sample, m.p. 79–80°, $[\alpha]_D$ +45.0° (*c* 1.85, dioxane), λ_{max}^{alc} 241 mμ, ϵ_{max} 15,100, which contained a quarter of a molecule of methanol of crystallization. Crystallization from other solvents was unsatisfactory.

Anal. Calcd. for $C_{28}H_{46}O \cdot {}^1/_4CH_3OH$: C, 83.43; H, 11.65. Found: C, 83.46; H, 11.30.

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(11) P. A. Plattner and A. Lang, *Helv. Chim. Acta*, **27**, 1872 (1944).

(12) D. H. R. Barton and W. H. Rosenfelder, *J. Chem. Soc.*, 1048 (1951).

(13) Melting points are corrected.

(14) Ushakov and Madaeva (reference 1) report a melting point of 215.5–216°.