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Cholesterol and Companions. I. Partial Dichromate Oxidation to Δ^4-Cholestene-6β-ol-3-one

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The oxidation of cholesterol with hexavalent chromium has been reinvestigated with use of solutions of sodium dichromate in benzene–acetic acid at temperatures from 0 to 87°. Mauthner and Suida's "α-oxycholestenol," readily obtainable in 16.5% yield, has been characterized as Δ^4-cholestene-6β-ol-3-one (II) by reduction with zinc and acetic acid to cholestenone, preparation by oxidation of Δ^4-chloestane-3β,6β-diol, and isomerization to cholestane-3,6-dione (V). The enolone II and the dione V both form yellow solutions of enolates in cold alcoholic alkali and II is not isomerized to V in the process. Acid isomerization of the acetate of II gave Δ^4-cholestene-6α-ol-3-one 6-acetate (VII), the structure of which was established by conversions paralleling those for the 6β-epimer. A product described by Rivett and Wallis differs from VII and must have some other structure. A further neutral product of oxidation obtainable from cholesterol in 7% yield is a 1:1 complex of II and epicholesterol. A mechanism for the epimerization of cholesterol through a 3,5-cyclic chromate is suggested. Several other 3-ketone 3-epi alcohol (α) complexes are described. It is shown that the first step in the production of II from cholesterol is formation of Δ^5-cholestene-3-one.

Interest in the possible existence of a non-aromatic steroid carcinogen derived from cholesterol[1,2] prompted this investigation of an already extensively studied theme. In their classical paper of 1896 on the oxidation of cholesterol with chromic acid, Mauthner and Suida[3] cite prior work extending back as much as fifty years on the oxidation of this readily available substance of obvious interest whose function in the animal organism is still a mystery. Oxidation of cholesterol with some of the subsequently studied reagents follows evident, normal paths: reaction with peracids to give the α-[4] and β-oxide,[5] dehydrogenation[6] or Oppenauer oxidation[7] to Δ^4-cholestene-3-one, *cis*-hydroxylation with permanganate[8] or with osmium tetroxide,[9] *trans*-hydroxylation with hydrogen peroxide[10] or with hydroxyl radicals.[11] Selenium dioxide[12] and air in colloidal solution[13] both effect allylic hydroxylation, but it is a curious fact that attack is at different allylic positions: 4β in the first case, 7α and 7β in the second. Oxidation of cholesterol with aqueous N-bromosuccinimide to cholestane-3β,5α-diol-6-one[14] presents another problem in interpretation.

The early results of Mauthner and Suida[3] raised some intriguing questions that have gone unanswered. These investigators isolated three neutral products, two of which were later recognized as Δ^4-cholestene-3,6-dione and cholestane-3,6-dione-5α-ol: the latter substance results also on oxidation with permanganate in acetic acid.[15] Windaus[16] characterized the first product by reduction with zinc and acetic acid to cholestane-3,6-dione and also isolated from the acidic fraction a substance identical with the Diels acid, prepared in 15% yield by oxidation of cholesterol with sodium hypobromite.[6,17] Ross[18] repeated the Mauthner–Suida oxidation and isolated the two neutral products just mentioned. The mechanisms of the oxidations leading to the two neutral products and to the Diels acid remain in doubt and the structure of the third neutral product is not known.

(1) L. F. Fieser, THIS JOURNAL, **73**, 5007 (1951).
(2) L. F. Fieser and W. P. Schneider, *ibid.*, **74**, 2254 (1952).
(3) J. Mauthner and W. Suida, *Monatsh.*, **17**, 579 (1896).
(4) T. Westphalen, *Ber.*, **48**, 1064 (1915).
(5) Z. Hattori, *J. Pharm. Soc. Japan*, **60**, 334 (1940) [*C. A.*, **34**, 7294 (1940)].
(6) O. Diels and E. Abderhalden, *Ber.*, **37**, 3092 (1904).
(7) R. V. Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).
(8) A. Windaus, *Ber.*, **40**, 257 (1907).
(9) M. I. Ushakov and A. I. Lutenberg, *Nature*, **140**, 466 (1937).
(10) R. H. Pickard and J. Yates, *J. Chem. Soc.*, **93**, 1678 (1908).
(11) G. R. Clemo, M. Keller and J. Weiss, *J. Chem. Soc.*, 3740 (1950); M. Keller and J. Weiss, *ibid.*, 2709 (1950).
(12) O. Rosenheim and W. W. Starling, *ibid.*, 377 (1937).
(13) S. Bergström and O. Wintersteiner, *J. Biol. Chem.*, **141**, 597 (1941); **143**, 503 (1942); **145**, 309, 327 (1942).
(14) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **71**, 3938 (1949).
(15) R. E. Marker and E. Rohrmann, *ibid.*, **62**, 516 (1940); M. Ehrenstein and M. T. Decker, *J. Org. Chem.*, **5**, 544 (1940), found that Δ^5-stenyl acetates are oxidized by permanganate in acetic acid to α- and β-oxides as well as to the 5α-ol-6-ones.
(16) A. Windaus, *Ber.*, **39**, 2249 (1906).
(17) O. Diels and E. Abderhalden, *ibid.*, **36**, 3177 (1903).
(18) W. C. J. Ross, *J. Chem. Soc.*, 737 (1946).

The first two papers of this series report a reinvestigation of the oxidation of cholesterol with hexavalent chromium; paper I describes oxidation with a limited amount of reagent, paper II with an excess. A departure from all procedures of oxidation known to me at the time was use of sodium dichromate dihydrate in acetic acid solution.[19] The dichromate is less hygroscopic than chromic anhydride and it dissolves in acetic acid without addition of water. Thus a 25% solution of dichromate in hot acetic acid can be cooled to 10° with no separation of solid. Cholesterol forms a sparingly soluble 1:1 complex with acetic acid,[20] the solubility of which at 25° is only 0.62 g. per 100 cc., and oxidation is conducted more conveniently by using one volume of benzene to two volumes of acetic acid. Thus solutions of 20 g. of cholesterol in 200 cc. of benzene and 25.6 g. or less of dichromate in 200 cc. of acetic acid are adjusted to any temperature from 10° up and mixed. With a large portion of dichromate at 10–25° a heavy paste of crystalline cholesteryl chromate separates and only slowly dissolves; with a small portion and at somewhat higher dilution, the solution after mixing can be cooled to 0° and the oxidation allowed to proceed at that temperature in homogeneous solution.

Mauthner and Suida characterized their third neutral product ("α-oxycholestenol") as melting at 180° (acetate, m.p. 102°) and showed that on oxidation it gives Δ⁴-cholestene-3,6-dione. This enedione is usually the main product of oxidation of cholesterol (see paper II), and cholestane-3,6-dione-5α-ol, although a minor product, is also easily isolated. In addition, I encountered two neutral products, m.p. 195° and 188°, and I believe that what Mauthner and Suida had in hand was the former contaminated with a little of the latter; the m.p. of the 195° product varies with the rate of heating and is strongly depressed by minute amounts of impurity. The substance was identified as Δ⁴-cholestene-6β-ol-3-one (II) by reduction with zinc and acetic acid, best as acetate, to Δ⁴-cholestene-3-one and by isolation of an identical product by dichromate oxidation of the known Δ⁴-cholestene-3β,6β-diol, as discussed further below. Further, the constants of the substance and its acetate agree with those reported for Δ⁴-cholestene-6β-ol-3-one[21] and not with those for Δ⁴-cholestene-3β-ol-6-one,[22] and the acetate did not depress the m.p. of a sample kindly supplied by Dr. Hans Reich and prepared according to Petrow by dehydration of cholestane-5α,6β-diol-3-one 6-acetate (IV). The enolone was characterized also by acidic isomerization to cholestane-3,6-dione (V) and by preparation of a 2,4-dinitrophenylhydrazone derivative. This derivative, as purified by crystallization, was red whereas that of cholestane-3,6-dione was yellow; however the red material showed a carbonyl band at 5.82 μ and it did not depress the m.p. of the slightly higher melting yellow product. The red contaminant probably is the derivative of Δ⁴,⁶-cholestadiene-3-one.

Δ⁴-Cholestene-6β-ol-3-one and cholestane-3,6-dione both share with Δ⁴-cholestene-3,6-dione the property of forming an enolate, but to a lesser extent. Thus when hexane solutions were shaken with equal volumes of Claisen's alkali the amounts extracted in the yellow alkaline layer were: Δ⁴-cholestene-3,6-dione, 81%; cholestane-3,6-dione, 59%; Δ⁴-cholestene-6β-ol-3-one, 48%. In each case the yellow solution showed low-intensity absorption in the region 260–266 mμ. However, the enolone and the dione do not form the same enol anion, since in the first case material recovered from the alkaline extract proved to be unchanged enolone. The alkaline isomerization of the enolone to the dione requires heating and probably does involve a common enolate. The nature of the low-temperature enolates is puzzling.

Herzig and Ehrenstein[23] discovered that a Δ⁴-ene-6β-ol-3-one 6-acetate of the allopregnane series could be epimerized at C_6 by hydrogen chloride in chloroform containing 0.7% ethanol (but not in pure chloroform). Application of their procedure to III gave Δ⁴-cholestene-6α-ol-3-one 6-acetate (VII). The structure was established by removal of the acetoxy group by reduction with zinc and acetic acid, which gave cholestenone, and by gentle saponification to the alcohol VI, which in turn was converted into cholestane-3,6-dione and Δ⁴-cholestene-3,6-dione. By the action of potassium acetate and acetic acid on 6β-bromo-Δ⁴-cholestene-3-one, Rivett and Wallis[24] obtained a substance that they assumed to have the structure VII. The present work shows this formulation to be in error and a reinvestigation of the product will be reported in a later paper of this series. The relationships between the 6-epimers in the cholestane series correspond well with those found by Herzig and Ehrenstein. Thus in each case the 6β-ol is less strongly dextrorotatory than the epimer; the M_D increment for epimerization of the 6β-hydroxyl compound is −218 in the cholestane series and −268 in the allopregnane series.

Communications from the Upjohn[25] and Squibb[26] research groups on the microbiological oxidation of progesterone to 11α-hydroxyprogesterone, a useful intermediate for production of cortisone, report the formation in about equal yield of a waste by-product characterized by the Squibb group as a 6,11α-dihydroxyprogesterone. Comparison of the constants of the by-product with those of Δ⁴-cholestene-6β-ol-3-one leaves little doubt that the extraneous hydroxyl group at C_6 is β-oriented. The by-product melts 83° higher than 11α-hydroxy-

(19) W. C. Meuly, U. S. Patent 2,505,646 (1950) [*C. A.*, **44**, 6894 (1950)], describes oxidation of cholesteryl acetate to the 7-ketone with a variety of metal chromates in acetic acid and other organic solvents. P. B. Report 73,485, p. 1579, describes oxidation of aceanthrene to aceanthrenequinone with dichromate in acetic acid in the presence of ceric acetate.

(20) F. Hoppe-Seyler, *Jahresberichte*, 545 (1863).

(21) B. Ellis and V. A. Petrow, *J. Chem. Soc.*, 1078 (1939).

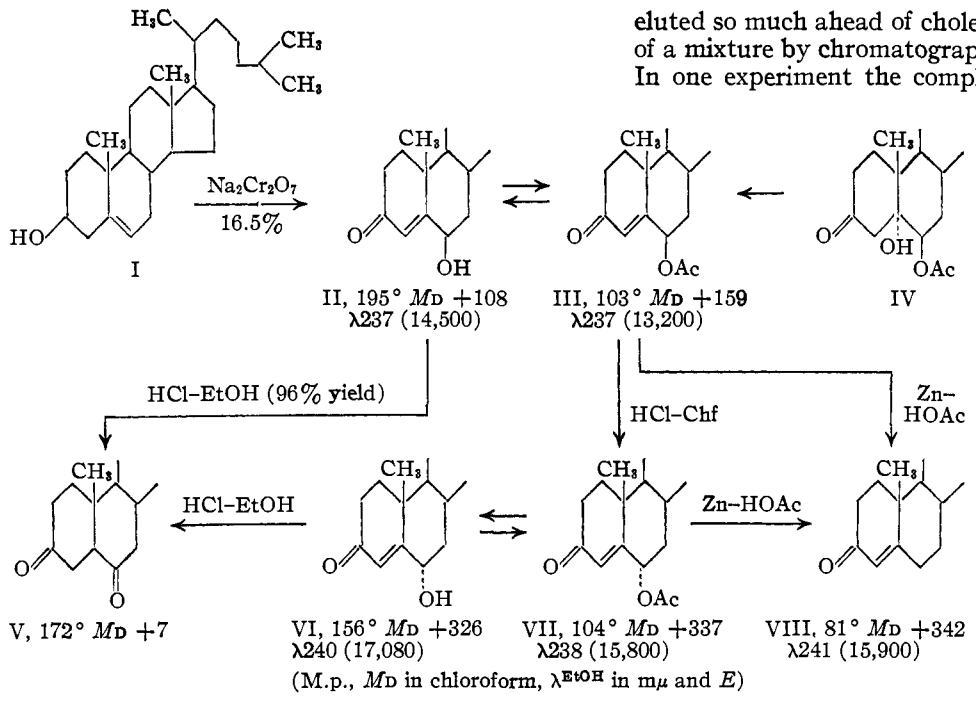
(22) I. M. Heilbron, E. R. H. Jones and F. S. Spring, *ibid.*, 801 (1937).

(23) P. Th. Herzig and M. Ehrenstein, *J. Org. Chem.*, **16**, 1050 (1951); see also C. P. Balant and M. Ehrenstein, *ibid.*, **17**, 1587 (1952).

(24) D. E. A. Rivett and E. S. Wallis, *ibid.*, **15**, 35 (1950).

(25) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1872 (1952). A later paper by Peterson, Murray, *et al.*, *ibid.*, **74**, 5933 (1952), describes the substance as the 6β,11α-derivative but presents no evidence of structure and configuration.

(26) J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *ibid.*, **74**, 3962 (1952).



(M.p., M_D in chloroform, λ^{EtOH} in mμ and E)

progesterone, the ultraviolet absorption maximum is shifted from 241 to 236 mμ, and the M_D increment for introduction of the 6-hydroxyl group is −244 Chf, Δ^4-Cholestene-6β-ol-3-one melts 144° higher than Δ^4-cholestene-3-one, the absorption maximum is shifted from 241 to 237 mμ, and the M_D effect of 6β-hydroxylation is −234; the M_D increment for 6α-hydroxylation is −16. The M_D effect of 6-acetoxylation of 11α-acetoxyprogesterone is −227; the M_D increments for 6β- and 6α-acetoxylation of Δ^4-cholestene-3-one are −183 and −5. The present finding that a 6α- or 6β-acetoxy substituent of Δ^4-cholestene-3-one can be eliminated smoothly by reduction with zinc dust and acetic acid suggested that the by-product of the microbiological oxidation might be convertible into the useful cortisone intermediate by the same method, and an experiment conducted at the Merck Laboratories under the direction of Dr. Max Tishler bore out this expectation.

The fourth neutral product of oxidation of cholesterol is a beautifully crystalline compound of sharp and constant melting point (188°). It seemed fully homogeneous and yet on acetylation or treatment with acids or bases it invariably gave two products. Acetylation afforded the acetates of Δ^4-cholestene-6β-ol-3-one and of an alcohol isomeric with cholesterol, treatment with acid or base gave cholestane-3,6-dione and the cholesterol isomer. Eventually the latter was recognized as epicholesterol, identified by comparison as alcohol and acetate, from the three-banded infrared acetate peak, and by conversion to epicholestanol and comparison with an authentic sample. The oxidation product is a 1:1 molecular complex of epicholesterol and Δ^4-cholestene-6β-ol-3-one. It can be split into the components very easily by chromatography on alumina, since epicholesterol is eluted by 1:1 petroleum ether–benzene whereas the enolone requires 3:1 benzene–ether. Epicholesterol is eluted so much ahead of cholesterol that separation of a mixture by chromatography should be feasible. In one experiment the complex was submitted to partial acetylation in ether–pyridine at 0°. The enolone was all converted to its acetate, whereas epicholesterol was found partly as acetate and partly as such; evidently the allylic 6β-hydroxyl group is more reactive than the 3α-hydroxyl group.

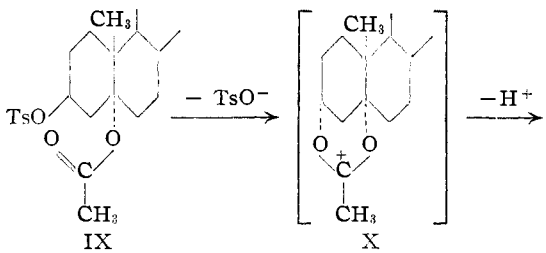
The complex is distinctly less soluble than either component and was obtained in 94% yield (two crops) from a solution of the components in ethanol. The specific rotation and extinction coefficient correspond to the values calculated. Tests with other pairs of components were made by dissolving equimolecular amounts in the minimum amount of hot ethanol or methanol and examining the crystallizate. If a complex forms it separates in high yield and purity, if not one or the other component separates in low yield and variable purity. Neither the 6β-hydroxyl group nor the double bond of the ketone component is necessary, since epicholesterol forms complexes with Δ^4-cholestene-6α-ol-3-one, with cholestenone, and with cholestanone, but not with coprostanone. However, Δ^4-cholestene-6β-ol-3-one is the best ketone component found; it forms a complex with epicholestanol whereas cholestanone does not. The same ketone fails to complex with cholesterol, cholestanol, coprostanol or epicoprostanol. In extending my observations, George H. Stout found that epicholesterol does not complex with 2-bromocholestane-3-one, pregnenolone, progesterone or testosterone, and that epismilagenin fails to complex with cholestanone.

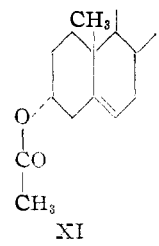
The requirements for complexing thus far defined suggest that in the complex the components are oriented back to back with the 3α-hydroxyl group of one component hydrogen-bonded to the 3-keto group of the other. However, comparison of the infrared spectra in Nujol mulls afforded evidence of only weak interactions.

The cholestenolone–epicholesterol complex was encountered first in early partial oxidations done at steam-bath temperature and was not found in parallel experiments done in the cold. The best procedure found for its preparation consists in adding a hot acetic acid solution of two oxygen equivalents of dichromate to a warm solution of cholesterol in benzene, when a green solution promptly results. An ethereal extract of the diluted mixture is washed neutral and concentrated to a point where the complex separates, and one

crystallization gives pure material in 7% yield. Since the alcohol component is easily isolated by chromatography, this constitutes a very simple method of preparing small batches of epicholesterol. The early inference that a high reaction temperature is required for formation of epicholesterol was later found to be wrong; the Experimental Part reports isolation of the complex epicholesterol-cholestenone from an oxidation (1.1 oxygen equiv.) conducted at 9° for 20 hr.

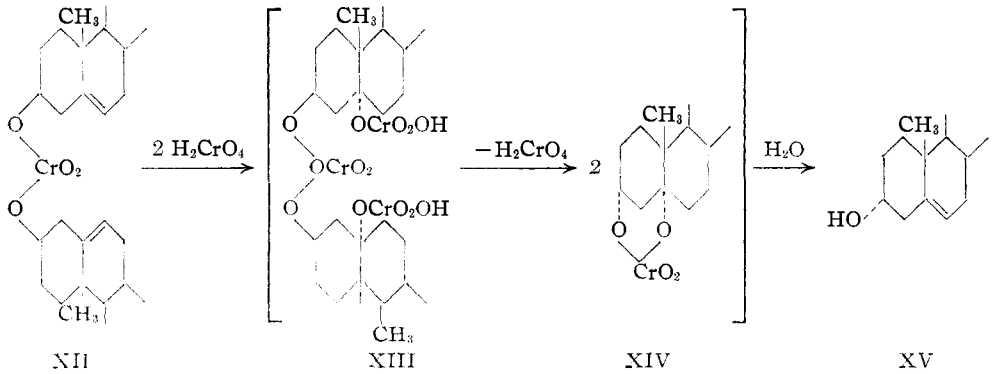
That two of the products of oxidation have unattacked secondary alcoholic groups is not of much significance, since they are products of partial oxidation and are often accompanied by considerable unchanged cholesterol. The surprising fact is that cholesterol is converted in part into epicholesterol. The double bond seems to be involved, since partial oxidation of cholestanol under the same conditions resulted in no epimerization. Most likely the reaction involves a 3α,5α-cyclic intermediate similar to that (X) postulated by Plattner and Lang[27] in explanation of the





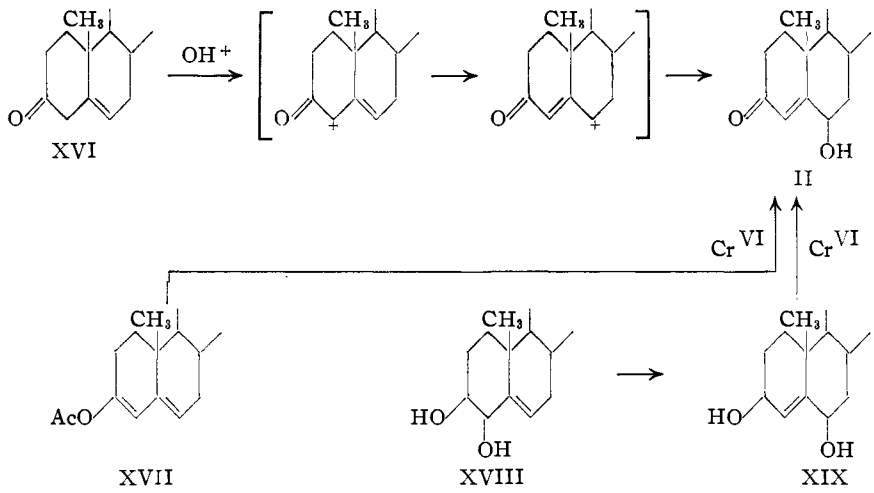
solvolysis of cholestane-3β,5α-diol 3-tosylate-5-acetate (IX) to epicholesteryl acetate. The key intermediate in the present case would be the cyclic chromate XIV, formed with inversion at C_3. The crystalline precipitate often initially observed is undoubtedly the normal chromate XII. Dr. D. H. R. Barton has suggested that the reaction initiating inversion is addition of chromic acid to the double bond to give XIII, which then loses chromic acid with cyclization to XIV.

The first step in the oxidation of cholesterol to Δ⁴-cholestene-6β-ol-3-one, beyond chromate formation, was established as follows. Chromatography of mother liquor material from partial oxidations afforded considerable amounts of cholestenone, but since this substance is stable to conditions of oxidation far more drastic than those employed it must have come from isomerization of Δ⁵-cholestene-3-one on the acid-washed alumina. In one preparation of the enolone–epicholesterol complex the mother liquor material was chromatographed on alumina more rapidly than usual and afforded in 20% yield unisomerized Δ⁵-cholestenone (and 36% of unchanged cholesterol). Furthermore in numerous exhaustive oxidations described in succeeding papers of the series the mixture was worked up by a process that would detect a mere trace of the conjugated ketone and, in properly conducted experiments, none was found. Therefore the Δ⁵-ketone is the initial product of oxidation and it does not undergo isomerization in acetic acid–benzene (at 0–25°, for periods up to 20 hr.). Partial oxidation of the pure Δ⁵-ketone (XVI), as expected, gave Δ⁴-cholestene-6β-ol-3-one (II). The same product also was formed on similar oxidation of cholestenone enol acetate (XVII) and of Δ⁴-cholestene-3β,6β-diol (XIX).[28] That this diol is attacked at C_3 rather than at C_6 is extraordinary, since cholestane-3β,5α,6β-triol is attacked by aqueous N-bromosuccinimide exclusively at C_6 and affords cholestane-3β,5α-diol-6-one in 96.5% yield.[15] That any of the Δ⁴-cholestene-6β-ol-3-one formed from cholesterol arises *via* allylic hydroxylation to XVIII, rearrangement (XIX) and oxidation, seems ruled out since no trace of either diol was found in any of numerous partial oxidations and since the allylic rearrangement requires temperatures far higher than those here involved. Possibly the oxidation of XVI to II proceeds *via* the carbonium ions written in brackets; however, no explanation has yet been found for the fact that the reaction is stereospecific and results in a 6β-oriented hydroxyl group, shown by the rearrangement reported above to be less stable than a 6α-hydroxyl group.

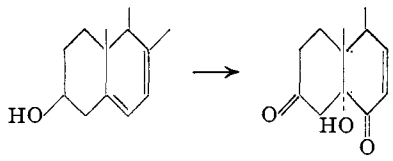


(27) Pl. A. Plattner and W. Lang, *Helv. Chim. Acta*, **27**, 1872 (1944).

(28) Drs. Franz Sondheimer and George Rosenkranz kindly sent me a copy of their manuscript "Steroids XL," *Experientia*, **9**, 62 (1953), reporting the smooth oxidation of XIX to II with manganese dioxide. In those points at which their work and mine overlap the results are in good agreement.



There remains to be mentioned isolation of minute amounts of three additional neutral oxidation products. One, eluted along with Δ^4-cholestenone, was encountered on four occasions and was finally identified as the complex of Δ^4-cholestenone with epicholesterol. The other two were isolated in the same early experiment from a now unidentifiable lot of cholesterol and have never been encountered since. Single analyses of each suggest the formulas $C_{27}H_{46}O$, m.p. 123°, α_D −40.4°, λ^{Chf} 5.81 μ; and $C_{27}H_{42}O_3$, m.p. 130°, α_D −32.4°, λ^{Chf} 5.80 μ. That the apparently more highly oxygenated substance is not derived from oxidation of 7-dehydrocholesterol (XX) was established by



XX XXI, m.p. 242° α_D +67° λ^{EtOH} 249 mμ (13,800)

oxidation of XX and isolation of an isomeric substance of properties consistent with formula XXI since the constants are similar to those of $\Delta^{7,22}$-ergostadiene-3,6-dione-5α-ol: m.p. 254°, α_D + 45° Chf, λ^{EtOH} 249 μ (11,600).[29]

Acknowledgments.—This work is part of a program sponsored by the National Cancer Institute and the American Cancer Society. I am particularly indebted to Research Corporation for a grant that contributes to my participation in the experimentation. I also wish to thank Koji Nakanishi and Wei-Yuan Huang for determination of optical constants.

Experimental

Δ^4-Cholestene-6β-ol-3-one.[30] **(a) From Cholesterol.**—This substance, which corresponds to Mauthner and Suida's "α-oxycholesterol"[3] in composition, m.p. of the acetate (M., S.: 101–102°) and, approximately, in m.p. (M., S.: 180°) was encountered first in an oxidation conducted by cooling a solution of 5 g. of cholesterol (crystallized once from acetic acid) in 200 cc. of acetic acid to 20°, adding a cold solution of 5.1 g. of sodium dichromate dihydrate (4 atoms of oxygen) n 50 cc. of acetic acid to the fine suspension of the acetic acid complex, and keeping the temperature at 16–20° by cooling. The solid soon dissolved and after 5 hr. the brown-green solution was diluted with water and the mixture extracted with ether. (No difference was noted in a parallel oxidation in which 10 cc. of *t*-butyl alcohol was added with the cholesterol). The neutral fraction on crystallization from methanol afforded 1.35 g. of **Δ^4-cholestene-3,6-dione**, m.p. 117–119.5°. The mother liquor was concentrated, cooled, scratched, and eventually yielded 1.4 g. of yellow solid, m.p. 123–140°. On digestion of this with 60–90° ligroin, a part remained undissolved and this when crystallized from methanol yielded 30 mg. of plates of **cholestane-3,6-dione-5α-ol**; when heated alone or in admixture with an authentic sample, the material softened at 228° and melted at 237°.

Concentration of the ligroin mother liquor gave 0.4 g. of a white powder, m.p. about 182°, which on crystallization from 30–60° ligroin (moderately soluble, hot) gave flat needles, m.p. 183–188° (varying with rate of heating), $\alpha^{23}D$ +27 ± 1° Chf, + 31 ± 1° Di, λ^{EtOH} 236 mμ (14,500).

Further purification was accomplished by conversion to the **acetate** (1 g. of alcohol in 5 cc. of pyridine and 2.5 cc. of acetic anhydride, 4 hr. at 25°), which was eluted from acid-washed alumina by benzene–petroleum ether (50:50) in a principal fraction, m.p. 100.5–101.5°. Crystallization from methanol gave large, heavy spars, m.p. 102–103°, λ^{CS_2} 5.75, 5.89 μ.

Anal. Calcd. for $C_{29}H_{46}O_3$ (442.66): C, 78.68; H, 10.48. Found: C, 78.90; H, 10.38.

The best sample of **Δ^4-cholestene-6β-ol-3-one** was obtained by saponification (probably partial) conducted for a brief period to avoid isomerization to cholestane-3,6-dione. A solution of 190 mg. of the pure acetate in 4 cc. of absolute ethanol was treated at 25° with 8 cc. of a solution of 0.3 g. of potassium hydroxide in 100 cc. of absolute ethanol. The solution soon became pale yellow; after 20 minutes it was diluted and acidified and the white solid precipitate when crystallized from ligroin gave 80 mg. of flat needles, m.p. 193–194° (introduced to the bath at 185°), $\alpha^{23}D$ +26.7 ± 0.3° Chf.

Anal. Calcd. for $C_{27}H_{44}O_2$ (400.62): C, 80.94; H, 11.07. Found: C, 81.07; H, 11.13.

In another experiment 400 mg. of acetate in 8 cc. of ethanol was let stand with 20 cc. of the potassium hydroxide solution for 30 min.; crystallization from ligroin gave 296 mg. of needles, m.p. 191.5–192.5° (introduced at 180° or at 190°).

Oxidation Procedure A.—The highest yields of Δ^4-cholestene-6β-ol-3-one were obtained by oxidation of 20 g. of cholesterol, purified either by crystallization from acetic acid or through the dibromide, as follows. A solution of the sterol in 100 cc. of benzene at 25° was diluted with 100 cc. of acetic acid, quickly cooled to 20°, and treated with a solution, also cooled to 20°, of either 10.2 g. (2 oxygen equiv.) or 15.3 g. (3 oxygen equiv.) of sodium dichromate dihydrate in 50 cc. of acetic acid, when a thin paste of orange cholesteryl chromate separated. The mixture was kept at 10–20° for about 4 hr., and then let come to room temperature and stand overnight. In the second experiment the total acid fraction amounted to 2.74 g. The neutral fraction was digested with 100 cc. of petroleum ether, the mixture cooled, and the pale yellow solid collected (4.3 g. and 4.3 g., m.p. 175–178°). Crystallization from about 350 cc. of hexane afforded 3.51 g. and 3.35 g. (16–17%) of white flat needles of the Δ^4-ene-6β-ol-3-one, m.p. 184–185°, and 189–191°. In the first experiment chromatography of the mother liquors established the presence of cholesterol.

(b) From Cholestenone Enol Acetate.—A solution of 1 g. of cholestenone enol acetate,[31] m.p. 79–80°, in 25 cc. of acetic acid at 25° was treated with a solution (25°) of 210 mg.

(29) W. E. Rosen, Dissertation, Harvard University (1952).

(30) At the time of submission of a communication[1] I was led through a faulty analysis to describe the substance as "a cholestenedione."

(31) Prepared by Miguel A. Romero.

(1 atom of oxygen) of sodium dichromate dihydrate in 5 cc. of acetic acid. The color changed to pure green in about 10 min., when water was added and the product collected by ether extraction. On digestion with petroleum ether, a white solid remained undissolved and was collected; 225 mg., m.p. 190°. The substance is fairly soluble in methanol but crystallized in long spars (slow crystallization) or in plates (recrystallization, faster), m.p. 193–194°, $\alpha^{21}D$ +28.4 ± 0.6° Chf, λ^{EtOH} 237 mμ (13,200).

Anal. Calcd. for $C_{27}H_{44}O_2$ (400.62): C, 80.94; H, 11.07. Found: C, 80.81; H, 11.07.

The yield was very much the same when the oxidation was conducted at 0° for 22 hr. (with addition of 10 cc. of benzene), at 90°, or with 1.5 oxygen equivalents.

A sample of material adsorbed on alumina from benzene was not eluted by a large volume of benzene but was promptly eluted by benzene–ether (50:50). Crystallization from 30–60° ligroin gave flat needles, $\alpha^{24}D$ +26.1 ± 0.1° Chf. The m.p. varied as follows with the temperature of the bath (given in parentheses): 176–177° (150°), 192–193° (180°), 194–195° (193°). Mixtures with starting material behaved the same way.

Anal. Calcd. for $C_{27}H_{44}O_2$ (400.62): C, 80.94; H, 11.07. Found: C, 80.90; H, 11.16.

The **acetate** formed slender needles from methanol, m.p. 101.5–102.5°, λ^{EtOH} 237 mμ (12,600).

Anal. Calcd. for $C_{29}H_{46}O_3$ (442.66): C, 78.68; H, 10.48. Found: C, 78.70; H, 10.42.

(c) **From Δ⁴-Cholestene-3β,6β-diol.**—Acetic acid (100 cc.) preheated to 90° was poured onto 2 g. of the diol,[31] m.p. 250–255°, and the mixture was heated quickly to the boiling point to bring the diol into solution and then treated with a solution of 544 mg. of sodium dichromate dihydrate in 5 cc. of acetic acid. The solution turned green at once and was promptly cooled. No crystals separated, and hence the solution was diluted and extracted with ether. A solution of the neutral fraction in about 20 cc. each of ether and petroleum ether on standing overnight at 4° deposited 0.43 g. of product, m.p. 175–178°. Crystallization from dioxane–methanol–water raised the m.p. to 179–181° (0.27 g.). In further crystallizations from ligroin, small amounts of sparingly soluble starting material were removed and the main product was then obtained as flat needles, m.p. 188.5–190°; no depression with sample (b); a mixture with the complex described below was depressed to 179–182°.

(d) **From Δ⁵-Cholestene-3-one.**—A solution of 1.13 g. (2.2 oxygen equiv.) of sodium dichromate dihydrate in 25 cc. of acetic acid was heated to 90° and poured on 2 g. of Δ⁵-cholestene-3-one,[32] m.p. 127°, $\alpha^{25}D$ +9.1° Chf. The resulting brown solution was kept above 80° and turned green in 2–3 minutes. The neutral fraction of the ether extract when digested with petroleum ether gave 0.31 g. of a tan solid that on clarification in ether, crystallization from ethanol (moderately soluble, slender flat needles, m.p. 169–170°), and then from ligroin gave tufts of colorless needles, m.p. 171–172°, undepressed on admixture with **cholestane-3,6-dione** (below). The dione is probably formed by isomerization of cholestenolone in the hot reaction mixture. Chromatography of the petroleum ether mother liquor afforded 0.64 g. of crude Δ⁴-cholestene-3,6-dione (from methanol, m.p. 123–124°) and 0.29 g. of crude Δ⁴-cholestene-6β-ol-3-one, m.p. 173–176° dec.; clarification in ether and crystallization from ligroin gave flat needles, m.p. 185–189°; mixture with (b), m.p. 188–191°.

In another experiment cooled solutions of 0.5 g. of Δ⁵-cholestene-3-one in 5 cc. of benzene and of 0.5 g. of sodium dichromate dihydrate in 20 cc. of acetic acid and 10 cc. of benzene were mixed and the solution cooled quickly to 0° and kept at this temperature overnight. Chromatography of the reaction product afforded Δ⁴-cholestenone (m.p. and mixed m.p. 81–82.5°), Δ⁴-cholestene-3,6-dione (m.p. 121–122°; no depression), and crude Δ⁴-cholestene-6β-ol-3-one, m.p. 173–175°, identified by infrared spectrum.

Oxidation was also accomplished by adding a solution of 8 g. of selenous acid in 2 cc. of water and 100 cc. of acetic acid to a solution of 10 g. of Δ⁵-cholestene-3-one in 50 cc. of benzene. The solution turned pink at once and after standing overnight at 25° was very dark and had deposited considerable selenium. The dark ether extract of the diluted mixture when washed with bicarbonate solution and then alkali deposited more selenium. The oil left on evaporation of solvent when digested with petroleum ether gave 3.3 g. of brown solid, m.p. 172–176°. Several crystallizations from hexane, methanol and methanol–water gave colorless blades, m.p. 188–189°, $\alpha^{22}D$ +35 ± 2° Chf, λ^{EtOH} 237 mμ (13,200), λ^{Chf} 2.8, 5.93, 6.00, 6.15 μ (found: C, 80.98; H, 11.14). The substance appeared to be Δ⁴-cholestene-6β-ol-3-one contaminated with a dextrorotatory impurity. Acetylation and crystallization three times from methanol gave the pure acetate, as long needles, m.p. and mixed m.p. 101–102° (found: C, 78.87; H, 10.48).

Reduction of Δ⁴-Cholestene-6β-ol-3-one 6-Acetate to Cholestenone.—A sample of acetate (m.p. 98–99°) made by acetylation of 0.5 g. of enolone derived from cholesterol by low-temperature oxidation was refluxed with 0.5 g. of zinc dust in 10 cc. of acetic acid for 15 hr. Extraction with ether and crystallization from methanol gave 290 mg. of large needles, m.p. 80–81°; mixed m.p. with cholestenone, λ^{CS_2} 5.94, 6.15 μ, 80.5–81.5°.

Anal. Calcd. for $C_{27}H_{44}O$ (384.63): C, 84.31; H, 11.53. Found: C, 84.08; H, 11.48.

Free Δ⁴-cholestene-6β-ol-3-one (1 g.) was refluxed with zinc and acetic acid in the same way. A total of 56 mg. of cholestane-3,6-dione separated when the ethereal extract was concentrated (recrystallized, m.p. 169–171°, mixed m.p. 170–172°). Chromatography of the mother liquor material afforded 78 mg. of cholestenone, m.p. 80–81°, mixed m.p. 80–82°.

Reduction of 6β,11α-Dihydroxyprogesterone to 11α-Hydroxyprogesterone (Experiment by Dr. Erwin F. Schoenewaldt of Merck and Co., Inc.).—A mixture of 52 mg. of 6β,11α-dihydroxyprogesterone, obtained by the oxidation of progesterone with a species of the fungus *Rhizopus*, 100 mg. of zinc dust and 3 cc. of glacial acetic acid was refluxed for 2¼ hr. and the mixture was cooled, diluted with 5 cc. of water, and extracted with two 5-cc. portions of chloroform. The extract was washed with 3 cc. of water, dried over anhydrous sodium sulfate, evaporated, and a trace of acetic acid removed in vacuum. A solution of the residue in 0.3 cc of warm ethyl acetate on cooling deposited white crystals, m.p. 162–166°, $\alpha^{23}D$ +172.5° Chf (*c* 0.374). The constants correspond to those of 11α-hydroxyprogesterone and comparison of the infrared spectrum of that of an authentic sample established identity.

Reaction of Δ⁴-Cholestene-6β-ol-3-one with 2,4-Dinitrophenylhydrazine.—Addition of two drops of 36% hydrochloric acid to a hot ethanolic solution of 0.4 g. of Δ⁴-cholestene-6β-ol-3-one and 198 mg. of 2,4-dinitrophenylhydrazine caused prompt separation of a red product, and after 5 min. the paste was filtered to give 0.52 g. of product, m.p. about 225°. This was crystallized from dioxane–ethanol (mat of fine red needles, m.p. 236–237°) and then eluted from alumina with benzene (leaving a small ring of reagent). Attempted crystallization from benzene–hexane gave a gel; crystallization from dioxane–ethanol gave small aggregates of short red needles, m.p. 238.5–239.5°; λ^{Chf} 3.00, 5.82, 6.12, 6.22, 6.60, 7.45 μ.

Anal. Calcd. for $C_{33}H_{48}O_5N_4$ (580.75): C, 68.24; H, 8.33; N, 9.65. Found: C, 68.15, 68.09; H, 8.19, 8.41; N, 9.36. This substance did not depress the m.p. of **cholestane-3,6-dione-2,4-dinitrophenylhydrazone** (see below).

Δ⁴-Cholestene-6β-acetoxy-3-one-2,4-dinitrophenylhydrazone.—Addition of 0.2 cc. of 36% hydrochloric acid to a solution of 1.2 g. of Δ⁴-cholestene-6β-ol-3-one acetate and 0.54 g. of 2,4-dinitrophenylhydrazine in 70 cc. of 95% ethanol caused prompt separation of yellow product. After digestion for 1–2 min. the mixture was cooled and the product collected (1.86 g. of bright orange-yellow powder). The substance crystallized from dioxane–methanol in small round aggregates, m.p. 165–167°. A further crystallization was done from acetone (readily soluble) with a little acetic acid added to keep the solution yellow (otherwise it turns deep red-yellow), but poorly formed granules were obtained, m.p. 164–166°. Product of somewhat better appearance resulted on crystallization from hexane (sparingly soluble) with enough benzene added to effect solution. This gave yellow microcrystals, m.p. 168–171°, λ^{EtOH} 260, 377 mμ (15,700, 30,500), λ^{Chf} 3.00, 5.78, 6.16, 6.25, 7.90 μ.

Anal. Calcd. for $C_{35}H_{50}O_6N_4$ (622.78): C, 67.50; H, 8.09. Found: C, 67.57; H, 8.17.

Enolate Ions. (a) **Δ⁴-Cholestene-6β-ol-3-one.**—A solution of 90 mg. of material, m.p. 191.5–192.5°, in 20 cc. of 30–60°

(32) Prepared by Xorge Alejandro Dominguez.

ligroin was shaken for 5 min. with 20 cc. of Claisen alkali[33] and the bright yellow lower layer was drawn off, diluted with water, and acidified (colorless). The extraction was repeated four times, by which time the yellow color of the alkaline extract had become faint. Ether extraction of the combined acidified extracts and crystallization from 30–60° ligroin afforded 30 mg. of blades, m.p. 184–186°; mixed m.p. with starting material, 188–191°.

In an analytical experiment[34] 12.8 mg. of Δ^4-cholestene 6β-ol-3-one in 2 cc. of 30–60° ligroin was shaken for 30 minutes with 2 cc. of freshly prepared Claisen's alkali. The ligroin layer on evaporation left 6.7 mg. of starting material (48% extraction). The Claisen's alkali extract showed a broad absorption maximum at 261 mμ (5,130).

(b) **Cholestane-3,6-dione.**—In a similar experiment[34] (7.4 mg.), an equal volume of Claisen alkali extracted 59% of the dione and the yellow extract showed a broad band at 260 mμ (4,470).

(c) **Δ^4-Cholestene-3,6-dione.**—The Claisen alkali extract removed 81% of the unsaturated diketone from 30–60° ligroin and the extract showed a broad plateau at 262–266 mμ (5,250).

(d) **Δ^4-Cholestene-3-one.**—In qualitative tests, a faint yellow color is observable in the Claisen alkali layer. In a quantitative experiment[34] with 12.6 mg. of material in 2 cc. of ligroin, the Claisen's alkali extract was only slightly colored and showed no ultraviolet absorption, and recovery in the ligroin solution (10.3 mg.) indicated no more than 18% extraction.

(e) **Δ^4-Cholestene-3β-ol-6-one 3-Acetate.**—A sample of this material, kindly supplied by Dr. H. Reich, was tested in parallel with a sample of Δ^4-cholestene-6β-ol-3-one 6-acetate by shaking solutions in 30–60° ligroin with Claisen alkali. In each instance the alkaline layer eventually developed a bright yellow color; the 3-ketone seemed to react more rapidly than the 6-ketonic isomer.

Δ^4-Cholestene-6α-ol-3-one Acetate.—As in one of the procedures of Herzig and Ehrenstein,[23] a solution of 0.6 g. of Δ^4-cholestene-6β-ol-3-one acetate, m.p. 102–103°, in 200 cc. of Merck chloroform (containing 0.7% ethanol) was cooled in an ice-bath and dry hydrogen chloride was bubbled in for 1.5 hr. The solution was washed with water and bicarbonate solution, dried and evaporated to a glass. On chromatography the material seemed homogeneous and was eluted by 50:50 petroleum ether–benzene but not at all by petroleum ether alone, which readily elutes the starting material. Nevertheless the substance was very slow to crystallize. It oiled out of methanol (very soluble)–water and failed to crystallize from petroleum ether (very soluble). However, when the glass was allowed to stand at 25° for a week a few crystals appeared around the carborundum boiling stone and on addition of enough petroleum ether to dissolve the oil and cooling a large crop of granular white crystals resulted; 221 mg. 102.5–103.5°, depression with starting material. The seeded mother liquor slowly deposited 150 mg. more material of the same m.p. The substance crystallized from methanol–water (usually only after scratching) in clusters of small prisms, m.p. 103–104°, $\alpha^{22}D$ +76.2 ± 0.3° Chf, λ^{EtOH} 238 mμ (broad), E 15,800; λ^{CS_2} 5.74, 5.91, 6.13 μ.

Anal. Calcd. for $C_{29}H_{46}O_3$ (442.66): C, 78.68; H, 10.48. Found: C, 78.73; H, 10.49.

In another experiment, with seed available, 4.7 g. of 6β-acetate gave 3.18 g. of 6α-acetate, m.p. 103–104°.

A 430-mg. sample of the α-acetate was refluxed overnight with zinc dust and acetic acid. The crude product failed to crystallize but when chromatographed yielded 116 mg. of cholestenone (long prisms from methanol, m.p. 80.5–81.5°, mixed m.p. 81–82°).

Δ^4-Cholestene-6α-ol-3-one.—A cold solution of 200 mg. of acetate in ethanol was treated with ethanolic potassium hydroxide, which at once gave a yellow solution, and let stand for 1 hr. On acidification and dilution the hydroxy compound separated as needles, m.p. 146–148° (150 mg.). It crystallized from 60–90° ligroin (sparingly soluble) in tufts of short needles, m.p. 154–156.5°; recrystallized, m.p. 155–156.5°, $\alpha^{23}D$ +81.3 ± 0.3° Chf, λ^{EtOH} 240 mμ (17,080).

Anal. Calcd. for $C_{27}H_{44}O_2$ (400.62): C, 80.94; H, 11.07. Found: C, 81.00; H, 11.25.

Dichromate oxidation of 160 mg. of enolone (3 hr., 25°) gave 110 mg. of Δ^4-cholestene-3,6-dione as yellow plates from methanol, m.p. and mixed m.p. 124–125°. For isomerization 108 mg. was refluxed for 2 hr. in 10 cc. of 95% ethanol containing 0.1 cc. 36% hydrochloric acid and the solution was diluted to saturation hot. Cholestane-3,6-dione separated as large needles, m.p. 170–171°, mixed m.p. 171–172°; yield 90 mg.

Ketone–Episterol Complexes. Complex of Δ^4-Cholestene-6β-ol-3-one with Epicholesterol. (a) By Oxidation of Cholesterol.—The best yield of the complex was obtained by dissolving 20 g. of Wilson cholesterol in 50 cc. of benzene, letting the solution cool to about 50°, and quickly adding a solution, preheated to 90°, of 5.6 g. of sodium dichromate dihydrate (2 oxygen equiv.) in 200 cc. of acetic acid. The solution turned green in a few moments and was cooled, diluted with water, and extracted with ether. The benzene–ether extract was washed with water three times, then with bicarbonate solution, dried and evaporated. Traces of benzene were removed by adding ether and evaporating, and then repeating the process. When the residue was dissolved in 50–60 cc. of ether and the solution let stand at 4°, the complex soon separated as a white solid that was collected and washed with ether; yield 2.11 g. (10.3%), m.p. 175–178°. Crystallization from 20 cc. of dioxane, 70 cc. of methanol and 5 cc. of water gave 1.50 g. (7.4%) of glistening plates, m.p. 186.5–187.5°.

The homogeneity of material crystallized from dioxane–methanol was tested by recrystallizing an 0.87-g. sample from 30–60° ligroin as follows: 1st recrystallization, m.p. 187.5–188°; 2nd, 188–189°; 3rd, 188–189°; a fourth recrystallization from dioxane–methanol gave plates, m.p. 188–188.5°. Concentration of the first mother liquor gave a second crop, m.p. 187.5–188°, and a third crop, m.p. 186–187°. The four-times recrystallized sample: $\alpha^{25}D$ −7.5 ± 0.2° Chf, λ^{Alc} 237 mμ (E 6,700), λ^{Chf} 2.80, 5.99, 6.23 μ. The extinction coefficient calculated for a 1:1 complex is E 6,700. A mixture with Δ^4-cholestene-6β-ol-3-one was depressed to 182–185°.

Anal. Calcd. for $C_{27}H_{44}O_2 \cdot C_{27}H_{46}O$ (787.26): C, 82.38; H, 11.52. Found: C, 82.16; H, 11.79.

The ethereal mother liquor remaining after filtration of the complex on evaporation gave 17.33 g. of glassy product, a 2.44-g. sample of which, when chromatographed rather rapidly, afforded 0.26 g. of Δ^5-cholestene-3-one (see below) and 1.03 g. of cholesterol (uncrystallized, m.p. 143–145°, mixed m.p. 144–147°), eluted by a 25:75 mixture of petroleum ether–benzene (but not by a 50:50 mixture). Thus the amount of cholesterol expended in the oxidation was 12.7 g., the yield of complex 16% (crude) or 11.5% (pure), and the yield of Δ^5-cholestene-3-one 20%.

The crude **Δ^5-cholestene-3-one**, eluted by a 75:25 petroleum ether–benzene mixture (not by petroleum ether alone), melted at 123–126°. Crystallization from 95% ethanol gave clusters of prismatic plates, m.p. 126–128°. Crystallized again, the material melted at 127–128°, and a mixture with an authentic sample melted at 125–126°.

Anal. Calcd. for $C_{27}H_{44}O$ (384.62): C, 84.31; H, 11.53. Found: C, 84.70; H, 11.78.

A 28-mg. sample was warmed with 2 cc. of methanol and one drop of 36% hydrochloric acid for 15 min. on the steam-bath and the solution diluted to turbidity and let stand, when needles of Δ^4-cholestene-3-one separated, m.p. 80.5–81.5°, mixed m.p. 81–82°.

When the oxidation was conducted in exactly the same way but with 3 oxygen equivalents of dichromate, the material separating from a concentrated ethereal solution of the neutral fraction amounted to 2.50 g. but was low melting (150–155°) and did not afford characteristic plates of the complex on crystallization from dioxane–methanol (or with added water). Several early oxidations were conducted by pouring an acetic acid solution (200 cc.) of 1 oxygen equivalent of dichromate, preheated to 90°, onto 20 g. of Wilson cholesterol. The green solution was cooled to 25° and scratched to cause separation of unchanged cholesterol as the acetic acid complex, which when dried afforded 9.4–10.4 g. of crude cholesterol, m.p. 140–144°. Ether extraction of the mother liquor and concentration of the neutral fraction gave 0.8–1.2 g. of crude complex, m.p. 165–170° or 0.6–0.8 g. of plates from dioxane–methanol. Cholesterol

(33) L. Claisen, *Ann.*, **418**, 96 (1915). A solution of 35 g. of potassium hydroxide in 25 cc. of water is diluted with 100 cc. of methanol.

(34) Conducted by Koji Nakanishi.

purified through the dibromide gave substantially the same results.

(b) **Acetylation of the Complex.**—Acetylation of 188° material in pyridine overnight at 25° gave an oily product that was separated by chromatography into two crystalline fractions. The first, eluted by petroleum ether, crystallized from methanol, in needles m.p. 85–86°, $\alpha^{23}D$ −14.5 ± 0.3° Chf, λ^{Chf} 5.82 μ, undepressed by admixture with **epicholesteryl acetate,**[35] m.p. 85–86°, $\alpha^{22}D$ −11.4 ± 0.4° Chf. Another sample, crystallized by dissolving it in a rather large volume of cold acetone and adding water to strong turbidity, formed needles, m.p. 86–87°, unchanged by further crystallization.

Anal. Calcd. for $C_{29}H_{48}O_2$ (428.67): C, 81.25; H, 11.29. Found: C, 81.29, 81.04; H, 11.30, 11.18.

A sample kindly examined by Dr. J. L. Johnson of the Upjohn Company through the courtesy of Dr. Arnold C. Ott showed the following pertinent absorptions: Nujol mull, 1730 cm.$^{-1}$ (C=O), 1258, 1236, 1226 cm.$^{-1}$ (C–O), 1021, 1010, 985 cm.$^{-1}$; chloroform solution, 1722 cm.$^{-1}$ (C=O), 1263, 1242 cm.$^{-1}$ (C–O). The three-peaked character of the strong band between 1250 and 1200 cm.$^{-1}$ is noteworthy, since Jones and co-workers[36] have shown that fine structure in this band distinguishes 3α- from 3β-acetoxy steroids.

The second fraction, eluted by 100% benzene (but not by 80:20 benzene–petroleum ether), was identified as **Δ^4-cholestene-6β-ol-3-one acetate.** Crystallized from methanol–water, it formed needles, m.p. 100–101°, $\alpha^{25}D$ +35.9 ± 0.8° Chf; undepressed on admixture with an authentic sample, m.p. 102–103°, supplied by Dr. H. Reich.

For saponification, 146 mg. of the acetate, m.p. 86–87° was dissolved in 6 cc. of 95% ethanol and the solution treated with 2 drops of 25% sodium hydroxide and warmed for 10 min. on the steam-bath and then diluted with water to the point of saturation and let cool. **Epicholesterol** separated in thin blades, m.p. 141.5–142.5° (116 mg.). One recrystallization from methanol (sparingly soluble) gave shiny, thin plates of constant m.p. 142–143°, $\alpha^{23}D$ −42.2 ± 0.3° Chf, λ^{Chf} 2.80 μ. The substance gives distinct m.p. depressions with both cholesterol and cholestanol.

Anal. Calcd. for $C_{27}H_{46}O$ (386.64): C, 83.87; H, 11.99. Found: C, 83.53; H, 12.21.

Hydrogenation[37] of 103 mg. of epicholesterol in 15 cc. of ethyl acetate with addition of 25 mg. of platinum oxide and 0.001 cc. of 71% perchloric acid[38] and crystallization from methanol gave 30 mg. of **epicholestanol,** m.p. 167–172°, negative to tetranitromethane. One recrystallization from methanol gave 20 mg., m.p. 181–182°, $\alpha^{22}D$ + 28 ± 0.05° Chf, undepressed by an authentic sample.

Anal. Calcd. for $C_{27}H_{48}O$ (388.65): C, 83.43; H, 12.45. Found: C, 83.66; H, 12.69.

Chromatography of the mother liquor material gave 15 mg. more material (eluted by 60:40 petroleum ether–benzene) m.p. 184–185°, mixed m.p. 185–186°.

In an early experiment 458 mg. of the 188° complex was let stand for 15 hr. at 0° in a solution of 10 cc. of pyridine, 6 cc. of ether and 5 cc. of acetic anhydride. Chromatography afforded 0.06 g. of epicholesteryl acetate, m.p. 84.5–85.5°, 0.16 g. of epicholesterol, m.p. 141.5–142.5°, and 0.10 g. of Δ^4-cholestene-6β-ol-3-one acetate, m.p. 99–101°. The result shows that the allylic 6β-hydroxy compound is acetylated more readily than epicholesterol.

(c) **Action of Acids and Bases on the Complex.**—A solution of 0.5 g. of complex in 10 cc. of acetic acid was refluxed for 19 hr. and the reaction mixture chromatographed. Petroleum ether eluted 0.15 g. of epicholesteryl acetate, m.p. and mixed m.p. 86–87°, and benzene eluted 111 mg. of **cholestane-3,6-dione,** m.p. 161–163°. This material, which is sparingly soluble in ether, very soluble in benzene or methanol, and moderately soluble in 30–60° ligroin, crystallized from the latter solvent in isolated tufts of needles, m.p. 171–172°, $\alpha^{22}D$ +1.8 ± 0.3° Chf, λ^{Chf} 5.82 μ. A mixture with an authentic sample, m.p. 171.5–172.5°, kindly supplied by Dr. Hans Reich, melted at 171.5–172.5°.

Anal. Calcd. for $C_{27}H_{44}O_2$ (400.62): C, 80.94; H, 11.07. Found: C, 81.15; H, 11.13.

Cholestane-3,6-dione was also formed when 290 mg. of complex was refluxed for 17 hr. with 4 cc. of pyridine and 2 cc. of acetic acid. Crystallization of the reaction mixture from ether and then from 30–60° ligroin gave 52 mg. of characteristic tufts of needles, m.p. 170–171°.

When 83 mg. of the complex was warmed in 10 cc. of 95% ethanol with 0.1 cc. of 36% hydrochloric acid for 1 hr. on the steam-bath and the mixture chromatographed and the chief fractions crystallized from methanol, 24 mg. of epicholesterol, m.p. 141–142°, and 10 mg. of cholestane-3,6-dione, m.p. 171–172°, were obtained. Treatment of 74 mg. in 10 cc. of 95% ethanol with 0.1 cc. of 25% sodium hydroxide for one-half hour on the steam-bath and chromatography afforded 18 mg. of cholestane-3,6-dione, m.p. 171–172° (from methanol) as the only isolated product.

(d) **Chromatographic Cleavage of the Complex.**—A solution of 1.57 g. of complex m.p. 185–186°, in petroleum ether–benzene (50:50), was chromatographed on a column of 50 g. of acid-washed alumina. Elution with the same solvent mixture gave three fractions totalling 0.70 g., m.p. 136°; crystallization from methanol gave 0.50 g. of **epicholesterol,** m.p. 141–142.5° (no depression in mixed m.p.). Benzene eluted only a trace of material, and a 3:1 mixture of benzene–ether eluted 0.60 g. of product, m.p. about 180°, identified as **Δ^4-cholestene-6β-ol-3-one** by conversion to the acetate, m.p. 102–103° (no depression in mixed m.p.).

(e) **Synthesis of the Complex.**—A hot solution of 53 mg. of Δ^4-cholestene-6β-ol-3-one (m.p. 194–195°, αD +26.7° Chf) in 2 cc. of 95% ethanol was treated with 51 mg. of epicholesterol (m.p. 141.5–142.5°, $\alpha^{23}D$ −42.2° Chf), when heavy precipitation occurred. On addition of 2 cc. more solvent, the solid was brought into solution at the b.p. On standing at 25° the complex soon began to separate in thin plates; collected after further cooling at 4°, the crystals amounted to 94 mg., m.p. 187.5–188.5°. Concentration gave a second crop of 8 mg., m.p. 186.5–187.5°. Recrystallization of the first crop did not change the m.p.; $\alpha^{23}D$ −7.5 ± 0.3° Chf (calcd. −7.6°); no depression on admixture with the sample (a).

Oxidation of Cholestanol with Insufficient Dichromate.—A solution of 232 mg. (0.5 oxygen equiv.) of dichromate in 20 cc. of acetic acid was heated to 90° and poured onto 2 g. of cholestanol. In about 15 sec. the material had all dissolved to a green solution. Chromatography of the neutral reaction mixture afforded a total of 0.74 g. of cholestanone (from methanol, m.p. 130–131°, no depression with an authentic sample), eluted by 75:25 petroleum ether–benzene, and 0.86 g. of cholestanol, m.p. in the range 142–144°.

Δ^4-Cholestene-6β-ol-3-one–Epicholestanol.—A solution of 53 mg. of the ketone and 51 mg. of epicholestanol, m.p. 185–186°, $\alpha^{22}D$ +25.5 ± 0.4° Chf, in 2 cc. of hot 95% ethanol on cooling to 25° deposited 80 mg. of thin plates, m.p. 179–180°. The material in the mother liquor was recovered by evaporation and on crystallization from methanol afforded 11 mg. of plates, m.p. 179–180°. Recrystallization of the top fraction did not change the m.p.; $\alpha^{22}D$ +25.7 ± 0.4° Chf (calcd. +27.0°). A mixture with epicholestanol was depressed to about 176°.

Anal. Calcd. for $C_{27}H_{44}O_2 \cdot C_{27}H_{48}O$ (789.27): C, 82.17; H, 11.75. Found: C, 82.27; H, 11.78.

Δ^4-Cholestene-6α-ol-3-one–Epicholesterol.—A mixture of 53 mg. of ketone and 51 mg. of alcohol was dissolved in 2 cc. of hot 95% ethanol and on standing at 4° deposited 45 mg. of plates, m.p. 161–162° (no change on recrystallization).

Anal. Calcd. for $C_{27}H_{44}O_2 \cdot C_{27}H_{46}O$ (787.26): C, 82.38; H, 11.52. Found: C, 82.22; H, 11.14.

Cholestenone[39]–Epicholesterol.—A solution of 70 mg. of each component in 4 cc. of hot methanol on cooling afforded 95 mg. of plates, m.p. 123–124°, and a second crop of 32 mg., m.p. 122.5–123.5. Recrystallization of the first crop: plates, m.p. 123–124°, $\alpha^{23}D$ +22.9 ± 0.3° Chf (calcd. +23.1°).

Anal. Calcd. for $(C_{27}H_{44}O \cdot C_{27}H_{46}O$ (771.26): C, 84.09; H, 11.76. Found: C, 84.10; H, 11.52.

(35) Prepared by E. J. Tarlton.

(36) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73,** 3215 (1951).

(37) Experiment by Wei-Yuan Huang.

(38) Compare E. B. Hershberg, E. Oliveto, M. Rubin, H. Staeudle and L. Kuhlen, THIS JOURNAL, **73,** 1144 (1951).

(39) Prepared by W. E. Rosen, m.p. 81–81.5°, $\alpha^{22}D$ +89° Chf, λ_{241}^{EtOH} (15,900). Cholestenone was recovered unchanged after treatment with excess dichromate in acetic acid at 80° for 2 hr.

Cholestanone–Epicholesterol.—Cholestanone (100 mg.), m.p. 130–131°, $\alpha^{31}D$ + 42.0 ± 0.3° Chf, was dissolved with an equal weight of epicholesterol in the minimal amount of hot methanol (5 cc.); the solution deposited 164 mg. of thin plates, m.p. 121–122° (unchanged on recrystallization), $\alpha^{23}D$ −1.2 ± 0.2° Chf (calcd. −0.2°).

Anal. Calcd. for $(C_{27}H_{46}O)_2$ (773.28): C, 83.87; H, 11.99. Found: C, 84.15; H, 11.80.

Dr. J. L. Johnson of Upjohn kindly compared the infrared spectrum of the complex with those of its components with the following results. Complex in Nujol mull: 1704 cm.$^{-1}$ (CO), 3430 cm.$^{-1}$ (OH); 3% solution in carbon tetrachloride: 3600, 1714 cm.$^{-1}$; cholestanone in Nujol, 1714 cm.$^{-1}$; in carbon tetrachloride: 1712 cm.$^{-1}$; epicholesterol in Nujol, 3330 cm.$^{-1}$, in carbon tetrachloride, 3600 cm.$^{-1}$. Dr. Johnson states: "The lower frequency regions of the Nujol mull spectra afford evidence of the formation of a new compound, namely, the complex. This evidence includes a number of shifts in band positions and also the occurrence of bands at frequencies at which neither component shows absorption. Two such bands occur at 1053 and 722 cm.$^{-1}$. The frequencies of the hydroxyl and carbonyl absorptions observed in the spectra of the solid samples fail to show evidence that these groups are involved in the formation of the complex."

Negative Trials.—Δ^4-Cholestene-6β-ol-3-one formed no complex with cholesterol, cholestanol, coprostanol or epicoprostanol. In the first two cases the alcoholic component separated first, in an impure condition in low yield, from a saturated solution of the components in 95% ethanol or in methanol; in the third and fourth instance the first crystallizate was the ketonic component. For example, a solution of 53 mg. of cholestenolone and 51 mg. of cholesterol in 2 cc. of 95% ethanol deposited 24 mg. of cholesterol, m.p. 146–148° (mixed m.p.). Δ^4-Cholestene-6β-ol-3-one acetate formed no complex with epicholesterol; coprostanone failed to complex with epicholesterol; cholestanone failed to complex with cholestanol or with epicholestanol. In the last case a solution of 70 mg. of each component in 8 cc. of hot methanol deposited 71 mg., m.p. 161–164°, and then 32 mg., m.p. 119–122°. Recrystallization of the first crop gave blades, m.p. 178–180°, undepressed by epicholestanol.

Unidentified Oxidation Products.—Oxidation of 20 g. of acetic acid-crystallized cholesterol with 20.4 g. of dichromate essentially as in procedure C at 20° for 6 hr. gave 17.9 g. of neutral material that after numerous crystallizations afforded 4.86 g. of Δ^4-cholestene-3,6-dione, 2.92 g. of Δ^4-cholestene-6β-ol-3-one and 40 mg. of cholestane-3β,5α-diol-6-one (the yield of the first product was about the same when the volume of the solvents was increased fourfold and the reaction conducted—in a homogeneous solution—at 0° for 20 hr.). Extraction of the combined mother liquors with Claisen alkali gave 0.98 g. more Δ^4-cholestene-3,6-dione. On chromatography of the dark residue (0.75 g.) on alumina (25 g.), the first solid fraction, eluted by 1:3 PE–B, crystallized from methanol (moderately soluble) to give short needles of product **A**, m.p. 122–123°, αD −40.4° Chf (*c* 0.70), λ^{Chf} 5.81 μ (no hydroxyl band); m.p. depressions with cholestanone and Δ^5-cholestene-3-one.

Anal. Calcd. for $C_{27}H_{46}O$ (386.64): C, 83.87; H, 11.99. Found: C, 83.72; H, 11.90.

Elution with benzene then gave four small crops of solid that were combined and crystallized from methanol, m.p. 108–115°. On further crystallization from methanol (sparingly soluble), product **B** separated as small plates, m.p. 129–130° (same on recrystallization), αD −32.4° Chf, λ^{Chf} 5.80 μ (no hydroxyl band).

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21. Found: C, 78,40; H, 10.60.

Further elution with benzene gave a total of 0.27 g. of cholesterol, m.p. and mixed m.p. 148.5–149.5°.

Δ^7-Cholestene-3,6-dione-5α-ol (XXI).—A solution of 1 g. of 7-dehydrocholesterol in 25 cc. of benzene was poured slowly into a mixture, cooled to 5°, of 30 cc. of benzene and a solution of 1 g. of dichromate in 75 cc. of acetic acid. The solution was cooled to 0° and kept at 0° for 15 hr. and worked up in the usual way. The neutral fraction was an oil that when rubbed with petroleum ether afforded 0.15 g. of white, high-melting solid. On two crystallizations from methanol (sparingly soluble) the substance formed lustrous thin plates, m.p. 240–242°, dec., αD +67° Chf (*c* 1.43), λ^{EtOH} 249 mμ (13,800), λ^{Chf} 2.81, 3.00, 5.85, 6.00, 6.19 μ.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21. Found: C, 78.28; H, 10.29.

Isolation of the Complex: Epicholesterol–Δ^4-Cholestene-3-one.—This substance was isolated on four occasions from the mixtures resulting from oxidation of 20 g. of cholesterol with 1.1 oxygen equivalents of dichromate for 20 hr. at 9° or with 4 equivalents at 20° for 1–6 hr. In the 1-hr. run the yields of other products were: acids, 0.84 g.; cholesterol, 6.4 g.; Δ^4- and Δ^5-cholestene-3-one, 4.98 g.; Δ^4-cholestene-3,6-dione, 2.4 g. In all instances the complex was found in the fraction eluted by 1:1 PE–B and was isolated from the mother liquor from crystallization of Δ^4-cholestene-3-one. The four samples, m.p. 122–123°, 121–122°, 120–121°, 120–121°, αD +24.8°, +20.5° Chf, λ^{Chf} 5.98, 6.17 μ, showed no depression in m.p. on admixture and one of them was compared with synthetic complex and did not depress the m.p. They depressed the m.p. of product A, above.

Anal. Calcd. for $C_{27}H_{44}O \cdot C_{27}H_{46}O$ (771.26): C, 84.09; H, 11.76. Found: C, 83.67, 83.99; H, 11.99, 11.79.

Cholestane-3,6-dione.—A convenient procedure was found in refluxing a solution of 3 g. of crude Δ^4-cholestene-6β-ol-3-one (m.p. 184–186°) in 50 cc. of 95% ethanol containing 1 cc. of 36% hydrochloric acid for 1.75 hr., when some crystals of the dione had already separated. The total crystallizate, after cooling, amounted to 2.67 g. (96%), m.p. 170–171° (see above for constants of pure material).

2,4-Dinitrophenylhydrazone.—A solution of 288 mg. of cholestane-3,6-dione and 142 mg. of 2,4-dinitrophenylhydrazine in 40 cc. of 95% ethanol was treated with 2 drops of 36% hydrochloric acid, warmed for 2 min. on the steam-bath, cooled, and the product collected; 274 mg., m.p. 240°. Crystallization from benzene gave masses of yellow crystal clusters, m.p. 241–242°, λ^{EtOH} 260, 360 mμ (7,090; 18,250); λ^{Chf} 3.00, 5.82, 6.12, 6.22 μ.

Anal. Calcd. for $C_{33}H_{48}O_5N_4$ (580.75): C, 68.24; H, 8.33. Found: C, 68.47; H, 8.51.

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