[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Cholesterol and Companions. VI. Lathosterol, Cholestanetriol and Ketone 104 from a Variety of Sources

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RECEIVED FEBRUARY 20, 1953

Lathosterol has been isolated from gallstone cholesterol after fractionation of the acetic acid and oxalic acid complexes; cholestanol was isolated from the same source. Cholestane-3β,5α,6β-triol was isolated from the cholesterol of gallstones, egg yolk and human brain, in the last case under conditions precluding the possibility that the triol is an artifact. Ketone 104 ($C_{27}H_{44}O_3$) was obtained from purified cholesterol of ten different sources in yields of 44–136 mg./20 g. A simple procedure is described for preparation of the ketone from unpurified cholesterol in lots of 300 g. to 30 lb. The ketone has been characterized by the preparation of seven crystalline derivatives. Reduction gives a liquid alcohol that affords a crystalline acetate. The alcohol is not precipitated by digitonin; since it is inert to bromine it cannot be the precursor of ketone 104. Two of the three oxygen atoms of ketone 104 and the alcohol appear to be present as oxide bridges and have resisted a number of attempts to effect cleavage under drastic conditions.

Since lathosterol has previously been isolated only from the cholesterol of cattle (paper III) and of animal skins,[2] we thought it desirable to determine if this companion is present in sterol of human origin. Gallstone sterol seemed a promising material both because of its ready availability and because the analyses of paper V have indicated a high average content of total Δ^7-stenol: 2.6%, as compared with 0.6% for Wilson Co. cholesterol. Colorless sterol of good quality was obtained in 77% yield without saponification from a large batch of pooled stones by digestion with hot dioxane, filtration from bile pigments and dilution with methanol and then water. In one experiment this sterol was crystallized twice from acetic acid as in paper III. Qualitative selenium dioxide tests showed the material from the first mother liquor to be much richer in Δ^7-stenol than that from the second, and, when the richer material was chromatographed as the acetate, pure lathosteryl acetate was isolated from late fractions; the course of the separation is readily apparent from micro selenium dioxide tests. The substance was isolated a second time in somewhat lower yield from the mother liquor resulting from crystallization of the 2:1 cholesterol–oxalic acid complex from hexane by a procedure kindly made available to us by Dr. Erwin Schwenk. A second crystallization of the bulk of the sterol as the oxalic acid complex gave material still containing 0.99% Δ^7-stenol.

The presence of cholestanol in gallstone cholesterol was established by the isolation of cholestanone after dichromate oxidation of material recovered from the first crystallization from acetic acid. The presence of a further companion substance was indicated by the result of chromatographic analysis of the sterol derived from the dioxane–methanol mother liquor from the processing of 150 g. of gallstones. Elution from alumina with 9:1 ether–methanol afforded a high-melting solid identified as cholestane-3β,5α,6β-triol by conversion to the 3,6-diacetate. The *trans*-triol was also isolated by a similar process from egg yolk cholesterol, which can be prepared conveniently by a process of simultaneous extraction and saponification conducted in a Soxhlet extractor with the boiling flask charged with 95% ethanolic potassium hydroxide. Through the courtesy of Dr. Max Tishler, a large batch of cholesterol was prepared for us at the Merck laboratories from commercial dried egg yolk. Cholestane-3β,5α,6β-triol was isolated also from the sterol extracted from human brain by mere digestion with acetone in a Waring blender at room temperature; we found that the formalized brain sections readily available from pathological laboratories constitute a very convenient source of brain sterol.

Cholestane-3β,5α,6β-triol has been isolated from ox liver extract,[3] from arteriosclerotic aorta,[4] and from the lipids of hog testes[5] but, in view of the ready formation of the triol from cholesterol by the action of hydroxyl radicals derived from a ferrous salt and hydrogen peroxide[6] or by the action of X-rays on water[7] it has seemed possible that the triol is an artifact arising from oxidation during saponification or at some other stage of the processing. We feel that the triol isolated from sterol extracted from brain by acetone at room temperature and without saponification can hardly be an artifact and hence that the triol is a true companion of cholesterol in brain tissue; the amount isolated is 0.5% of the total cholesterol isolated from this source. The triol found in the sterol extracted by hot dioxane from gallstones also is probably not an artifact.

We next investigated cholesterol from various sources for the presence of the precursor of ketone 104. The method of oxidative analysis of 20-g. samples described in paper III was applied with the results recorded in Table I. All samples of cho-

TABLE I

KETONE 104 FROM CHOLESTEROL PURIFIED THROUGH THE DIBROMIDE

Source	Mg./20 g.	Source	Mg./20 g.
Wilson Co., lot 71885	132	Pig spinal cord	44
Wilson Co., lot 69768	100	Whale liver oil	75
Gallstones	100	Shark liver oil	114
Egg yolk	100	Mollusk sterol (less	
Human brain	120	brassicasterol)	136
Pig brain	71	Wool fat	103

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(5) L. Ruzicka and V. Prelog, *ibid.*, **26**, 975 (1943).

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TABLE II

PROPERTIES OF KETONE 104 AND DERIVATIVES

Compound	M.p., °C.	α_D Chf	M_D	Infrared absorption (μ in Chf.)			
Ketone 104	123.5–124.5	−37.1°	−154	5.78	8.98	9.81	10.05	11.07
Alcohol 104	Oil	−22.5°	−94		8.98		10.05	11.01
Acetate	100–101.5	−32.6°	−150	5.76	8.95	9.78	10.05	11.05
2,4-Dinitrophenylhydrazone	182–184.4							
Ethylenethioketal	103–104	−23.4°	−150		8.98	9.81	10.05	11.05
Oxime	194.6–195.2							
Desoxo derivative	54.8–55.8	+2.9°	+11		8.98	9.5	(10.28)	(11.07)
Enol acetate	115.6–116	+15.9°	+73	5.86, 6.05			(10.28)	(11.1)
Lactone	89–90	+8.9	+38	5.80	8.8		10.05	11.15

lesterol thus far examined have yielded ketone 104 on oxidation, and no source appreciably better than commercial material from spinal cord and brain of cattle has been found.

The previously described method of preparing ketone 104 by exhaustive dichromate oxidation at room temperature, petroleum ether phase-separation, extraction with Claisen alkali, chromatography and partition with Grignard reagent (paper III) has now been further improved and standardized in detail. On a laboratory scale it is possible to obtain 2.6 g. of pure ketone 104 from 300 g. of commercial cholesterol in a brief working period. The yield of cholestanone simultaneously formed indicates the presence of about 0.07% of cholestanol in the starting material. Through the courtesy of Dr. Max Tishler a 30-lb. batch of Wilson Co. cholesterol was processed in the Merck pilot plant under the direction of Dr. Malcolm L. Brown, and no difficulty was encountered in the large-scale application of the laboratory procedure.

Chemical characterization of ketone 104 is rendered difficult by the inert character of two of the three oxygen atoms present. Thus the substance is unaffected by refluxing methanolic sulfuric acid or by phosphorus oxychloride in pyridine. Also, it is not oxidized by sodium hypobromite in dioxane and it is inert to bromine. That the ketonic function is less reactive than that of 3-ketosteroids is indicated by the already mentioned (paper III) differentiation from cholestanone in the rate of reaction with Girard reagent. However, the following ketonic derivatives have now been isolated in crystalline form: oxime, 2,4-dinitrophenylhydrazone, ethylenethioketal, enol acetate, lactone (with perbenzoic acid). The enol acetate resulted from treatment of ketone 104 with acetic anhydride and aluminum chloride at about 140°; that the two inert oxygen atoms remained undisturbed is shown by acid hydrolysis of the enol acetate to ketone 104. Reduction of ketone 104 with lithium aluminum hydride gave an alcohol that could not be obtained in solid form but that yielded a crystalline acetate. Saponification of the pure acetate gave alcohol that again was an oil; ketone 104 was regenerated from the oil by oxidation. The specific rotation was very nearly the same before and after purification through the crystalline acetate. The alcohol is not precipitated by digitonin and it shows no reaction with bromine under conditions of the precipitation of cholesterol dibromide. The latter observation shows that this alcohol is not the precursor of ketone 104 present in tissue cholesterol, since the precursor separates with cholesterol dibromide. Alcohol 104 was recovered unchanged (as acetate) after attempted hydrogenation in the presence of Raney nickel or Adams catalyst and after attempted Clemmensen reduction.

The analyses of ketone 104 and of the seven crystalline derivatives all agree closely with the theory for $C_{27}H_{44}O_3$. The formulas $C_{26}H_{42}O_3$ and $C_{28}H_{46}O_3$ might be regarded as admissible alternates on the basis of the analyses of the ketone itself, but are ruled out by the carbon percentages found for the ethylenethioketal and 2,4-dinitrophenylhydrazone derivatives, since here the homologs differ in carbon content by 0.42–0.46%. From the summary of properties given in Table II it will be seen that the infrared absorption associated with the carbonyl group disappears on reduction with lithium aluminum hydride, on condensation with ethanedithiol, and in the product of desulfurization of the latter derivative. Since there is no suggestion in the spectra of the presence of a hydroxyl group, the two remaining inert oxygen atoms must be present as oxide bridges. Four strong infrared absorption bands at 9, 9.8, 10 and 11.1 μ characteristic of ketone 104 (Fig. 1) appear in the spectra of nearly all derivatives. All of these bands are probably associated with oxide bridges; thus bands at 9μ and at 11μ are characteristic of ether and 1,2-oxide groups, respectively. The spectrum (Fig. 1) is not of the type characteristic of steroid sapogenins, and ketone 104 is fully resistant to reactions by which the sapogenin side chain can be opened.

The desoxo derivative of ketone 104 thus appears to be a saturated substance of the formula $C_{27}H_{46}O_2$ containing two bridging oxygen atoms. Such a substance would contain three carbocyclic rings and hence, if it is indeed related to a sterol at all, it would be related more closely to a secosterol like vitamin D_3 than to cholesterol. It may be significant that the hydro derivatives of vitamin D_3, like alcohol 104, are not precipitated by digitonin. The conversion of ketone 104 into any of the derivatives listed in Table II is attended with a dextrorotatory shift in M_D. The M_D increments for conversion to the alcohol, desoxo compound, enol acetate and lactone are +60, +165, +227 and +192. The corresponding values for cholestanone are −65, −67, −99 and −153; for coprostanone the shifts are +16, +0, +67 and +105. However, if ketone 104 has a sterol-like ring A with the

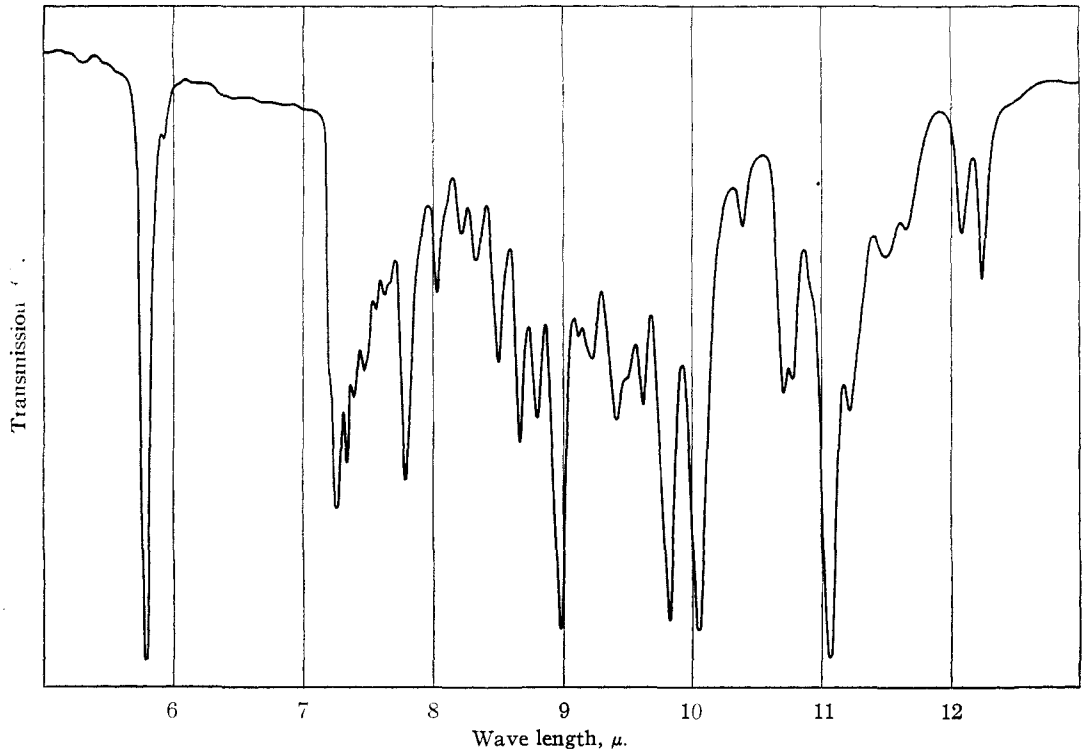

Fig. 1.—Infrared spectrum of ketone 104 in carbon bisulfide.

carbonyl group at C_3, the evidence of hindrance must mean that there is an oxide bridge in the neighborhood of C_3. This bridge would introduce another center of asymmetry, such as to invalidate M_D comparisons,

An X-ray analysis of ketone 104 kindly carried out by Dr. Ray Pepinsky and associates at Pennsylvania State College indicated the following unit cell dimensions: a = 22.0 Å., b = 7.3 Å., c = 20.5 Å.

Acknowledgments.—We are indebted to Dr. Max Tishler and Merck and Co., Inc., for preparation of ketone 104 and of a large batch of egg yolk cholesterol. We also thank Drs. Jacob Fine and Arnold M. Seligman of Beth Israel Hospital for supplies of gallstones and human brains, Dr. Werner Bergmann for mollusk sterol, and Drs. Alberto Ercoli and Pietro de Ruggieri of Vismara Terapeutica, Casatenovo, Italy, for cholesterol from pig brain and spinal cord.

Experimental

Gallstone Cholesterol. (a) **Preparation.**—A mixture of 239 g. of powdered gallstones and 1673 cc. of dioxane was heated on the steam-bath and the resulting solution filtered from a brown residue of bile pigments. The yellow filtrate was diluted with 1673 cc. of methanol and the solution clarified by Norit, filtered hot (weak greenish-yellow), and diluted with water to the point of saturation at the b.p. Cholesterol separated on cooling and after being washed with methanol consisted of pure white plates, m.p. 143–145°, yield 185 g. (77%).

(b) **Fractionation with Acetic Acid.**—The above crude sterol (185 g.) was crystallized twice from 8 cc./g. of acetic acid as described in paper III to give the fractions A_1, 166 g., and A_2, 152 g., m.p. 147–148°. The total mother liquor material from A_1 (ether extraction, saponification) amounting to 16.5 g., m.p. 115–127°, selenium dioxide test: yellow in 30 sec., red in 2 min.; that from A_2 (10.9 g., m.p. 127–137°): yellow in 2 min., red in 5 min.

Acetylation of the A_1 mother liquor sterol gave acetate m.p. 85–93°, 500 mg. of which was adsorbed on 110 g. of alumina and the column eluted with 100-cc. portions of petroleum ether. Fractions 6–16 melted in the range 92–108° and gave selenium dioxide tests of increasing intensity. Fractions 19–27 were solid and gave strong tests and two crystallizations of the combined material from methanol gave 9 mg. of **lathosteryl acetate**, m.p. and mixed m.p. 118–119°.

(c) **Fractionation with Oxalic Acid.**—A mixture of 69 g. of crude gallstone cholesterol, 70 g. of anhydrous oxalic acid, and 1050 cc. of redistilled hexane was refluxed for 1.5 hr., cooled, and the crystalline oxalic acid complex collected. This was decomposed with acetone and water, the sterol recovered, and the process repeated. Analysis of the twice purified sterol indicated a content of 0.99% Δ^7-stenol.

The sterol recovered from the mother liquor of the first crystallization of the complex (4.2 g.) after two crystallizations from methanol (1.42 g.) melted at 119–128°. A 1-g. sample was chromatographed on 30 g. of alumina as above. The first solid material appeared in fraction 18 (m.p. 100–107°), eluted by 9:1 benzene–ether. Fractions 24–31, eluted by the 9:1 mixture, m.p. 129–135°, were combined (0.44 g.), acetylated, and the acetate chromatographed (110 g. of alumina, petroleum ether). Fractions 6–10 (m.p. 97–106°) were selenium dioxide-negative and the next ones were increasingly strongly positive. The solid material from fraction 16 on two crystallizations from methanol afforded 4.7 mg. of **lathosteryl acetate**, m.p. 117–118°, α_D +1.9° Chf (c 4.2), mixed m.p. 117–119° (depressed by cholesteryl acetate to 93–98°). Fractions 14 and 15 on three crystallizations gave 13 mg. of acetate, m.p. 113–115°, that on saponification and four crystallizations from methanol gave 3 mg. of **lathosterol**, m.p. 121–122°, mixed m.p. 122–123°.

(d) **Isolation of Cholestanone.**—A solution of 5 g. of A_1 mother liquor sterol in 100 cc. of benzene and 150 cc. of acetic acid was cooled to 8° and treated with a cold solution of 12.8 g. of sodium dichromate dihydrate in 50 cc. of acetic acid. After 16 hr. at 7–9° the mixture was worked up as in procedure B, paper II. Chromatography of the Claisen alkali extraction residue gave 370 mg. of **cholestanone**, m.p. 124–129°, eluted by 9.5:0.5 petroleum ether–benzene. Recrystallization from methanol gave 293 mg., m.p. 129–130°, and two more crystallizations gave ketone, m.p. and mixed m.p. 129–130°, α_D +39.8° Chf (c 1.18).

Anal. Calcd. for $C_{27}H_{46}O$ (386.64): C, 83.87; H, 11.99. Found: C, 83.99; H, 11.45.

The weight of crude cholestanone indicates the presence of at least 0.66% of cholestanol in the crude gallstone cholesterol. A later fraction, eluted by a 10:1 solvent mixture, melted at 136–140° (30 mg.) and after three crystallizations from methanol melted at 142–143° and did not depress the m.p. of **lathostenone**.

(e) **Isolation of Cholestane-3β,5α,6β-triol.**—The dioxane–methanol mother liquor from extraction of another 150 g. of gallstones was evaporated on the steam-bath to a small volume and the residue extracted with ether. The recovered material was adsorbed on 200 g. of alumina and the column eluted with 1 l. of benzene (negative), 500 cc. of benzene–ether (oil), 1 l. of ether (oil) and then with 200-cc. portions of 9:1 ether–methanol. Fractions 1–6 and 10–16 were oils. Fractions 7–9 gave solids, m.p. 226–228°, which were combined and acetylated in pyridine (16 hr., 25°). The product, collected by ether extraction, was crystallized from methanol (m.p. 158–161°), and recrystallized after treatment with norit. This afforded 15 mg. of **cholestane-3β,5α,6β-triol 3,6-diacetate**, m.p. 163–164.5°, α_D −45.9° Chf (*c* 0.84), no depression on admixture with an authentic sample.

Egg Yolk Cholesterol. (a) **Preparation.**—A convenient process of isolation consists in extraction with alcohol and simultaneous saponification. Thus 60 g. of commercial dried egg yolk was placed in the thimble of a Soxhlet extractor and a mixture of 350 cc. of 95% ethanol and a solution of 15 g. of potassium hydroxide in 15 cc. of water was placed in the boiling flask and extraction continued for 14 hr. The solution was then diluted with 1.3 l. of water and extracted with three 200-cc. portions of ether (there is no trouble from emulsions when about 20% of ethanol is present and when the mixture is not acidified). Evaporation of the washed and dried extract gave 2.5 g. of residue consisting of solid and adhering oil. This material was crystallized from 95% ethanol (20 cc.) and the crystals washed with ethanol and then a little acetic acid to remove adhering coloring matter and give faintly yellow sterol, m.p. 146–148°; yield 1 g.

(b) **Isolation of Cholestane-3β,5α,6β-triol.**—The mother liquor sterol from crystallization of 74 g. of egg yolk cholesterol from acetic acid was collected by ether extraction after dilution with water and neutralization with alkali. Crystallization from ethanol gave 2.7 g. of cholesterol, m.p. 145–147°. Evaporation of the mother liquor left 9 g. of gummy residue, which was chromatographed as above on 180 g. of alumina. Elution with 9:1 ether–methanol and crystallization from methanol gave 18.8 mg. of cholestanetriol, m.p. 231–233° dec.; 3,6-diacetate, m.p. 166–167°, undepressed on admixture with authentic material.

Cholestane-3β,5α,6β-triol from Human Brain.—Formalized human brain (102 g.) was digested with acetone (200 cc.) in a Waring blender for 3 hr. at 25°, the mixture let stand for a few hours and filtered. The residue was extracted again with 200 cc. of acetone and the combined filtrate concentrated to ¾ its volume, filtered from a little gum and solid, and evaporated to dryness. Crystallization from methanol gave 0.77 g. of cholesterol, m.p. 146–147°.

A total of 1680 g. of human brain was processed in the same way and the mother liquor from the cholesterol evaporated and the residue chromatographed on 300 g. of alumina. The solid product from two of the 9:1 ether–methanol eluates when washed with benzene gave white material, m.p. 231–235° dec. Acetylation and crystallization twice from methanol gave 35 mg. of 3,6-diacetate, m.p. and mixed m.p. 166–167°, α_D −44.7° Chf (*c* 1.10). A further 26 mg. of impure diacetate was obtained from the mother liquor.

Ketone 104 from Cholesterol of Various Sources.—Twenty-gram batches of cholesterol were oxidized at 10–18° (running tap water) for 14–18 hr. by procedure B and the mixture processed as in paper III by ether extraction, extraction of the neutral fraction with Claisen alkali, and chromatography of the residue (0.8–1.5 g.). Δ^4-Cholestene-3-one was found present when the temperature of oxidation fell below 7°; it is eluted after ketone 104. Ketone 104 was eluted by petroleum ether containing 10–15% benzene. The results are recorded in Table I.

The oxidation of egg yolk cholesterol afforded an unidentified product eluted by 1:1 petroleum ether–benzene. Crystallized from methanol–chloroform, this melted at 200–201.2° (32 mg.), α_D −43.4° Chf (*c* 1.22), found; C, 85.30; H, 11.90.

Preparation of Ketone 104.—A mixture of 300 g. of Wilson Co. cholesterol lot 74078 and 2.4 l. of benzene was placed in a 12-l. round-bottomed flask fitted with a mechanical stirrer and thermometer and supported in a pan that later could be filled with ice and water, conveniently mounted on an automobile jack so that it could be raised or lowered as required for regulation of temperature. The mixture was stirred to effect solution of the cholesterol, and during the process 768 g. of sodium dichromate dihydrate was dissolved in 2.4 l. of acetic acid and the solution cooled to 15°. After this chilled solution of dichromate was ready, the benzene solution of cholesterol was cooled to 10°, 2.4 l. of acetic acid was added, the solution was cooled quickly to 15° to avoid acetylation and the chilled dichromate solution was added all at once. A considerable amount of orange cholesteryl chromate separated to give a fairly thick paste. The reaction mixture was stirred continuously and the temperature was maintained at 14–16° for a total of 5 hr. The chromate ester dissolved after about 1.5 hr. to a deep brown solution. After 5 hr. stirring was stopped and the solution let stand in the pan with an amount of ice present estimated to melt in 2–3 hr. Thus the solution was let come to room temperature and to stand overnight (16–18 hr.). The next morning (temperature of solution 30°) 3 l. of 30–60° petroleum ether was added to the brown solution and the mixture was stirred for 15 min. and allowed to settle to a large, light-colored upper layer and a small, deep red-brown lower layer (interface is observable under suitable illumination). The lower layer was removed by siphoning and discarded (3.4–3.7 l.). Then 600 cc. of water was added and the mixture stirred and allowed to settle. A lower layer of 2.1–2.3 l. was again siphoned off and discarded. The process was repeated with another 600-cc. portion of water (1–1.2 l. of lower layer removed) and then with 300 cc. of water (400–600 cc. of lower layer). Next 600 cc. of saturated aqueous sodium chloride solution was added, the mixture stirred, and the lower layer removed. The light colored benzene–petroleum ether solution, now free of chromium compounds and containing only a little acetic acid, was stirred vigorously with 1.2 l. of Claisen alkali[8] and the mixture let settle. A brown lower layer containing acidic oxidation products and Δ^4-cholestene-3,6-dione was siphoned off and discarded, and the process was repeated with 800 cc. of Claisen alkali. The remaining hydrocarbon layer was then treated with 50 cc. of Claisen alkali and evaporated to a volume of about 1.5 l.[9] The solution was then cooled to room temperature, transferred to a separatory funnel, and the small lower layer was drawn off and discarded. The hydrocarbon layer was then shaken vigorously with two 400-cc. portions of Claisen alkali and the rather weakly yellow extracts discarded. Then water was added, together with enough hydrochloric acid to neutralize adhering alkali; the mixture was shaken only gently prior to separation. The hydrocarbon layer was then filtered by gravity through magnesium sulfate and evaporated to dryness; last traces of benzene were removed by adding petroleum ether, evaporating, and evacuating at the water pump. The brown residual oil (11.5 g.) was treated with 240 cc. of methanol and the volume marked on the flask. Then 60 cc. more methanol was added and the mixture boiled on the steam-bath until the volume was reduced to 240 cc.; a small amount of undesired oily product remained undissolved. The mixture was then cooled to 25°, 0.5 g. of Norit was added, and the mixture was swirled for 2 min. and filtered by gravity. The filtrate was evaporated to dryness and the last traces of methanol removed by evaporation with 10 cc. of benzene and evacuation at the water pump.

The residual brown oil (10 g.) was dissolved in 1 l. of 30–60° petroleum ether and the solution passed through a column of 300 g. of acid-washed alumina. The column was eluted further with a 1-l. portion of petroleum ether and the eluate discarded. Then it was eluted with 4 l. of 80:20 pe-

(8) A solution of 3.5 kg. of potassium hydroxide in 2.5 l. of water is cooled to room temperature, 10 l. of methanol is added, and the solution again cooled to room temperature.

(9) The purpose of this operation is to saponify any cholesteryl acetate and to decrease the amount of Claisen alkali required for complete extraction.

troleum ether (30–60°)-benzene and the total eluate containing ketone 104 and cholestanone collected in one flask. Evaporation of the 80:20 eluate left a residue that solidified when rubbed with 10 cc. of methanol. The resulting suspension of white solid was transferred with 130 cc. of ether to a 500-cc. Erlenmeyer flask and the solution treated with 130 cc. of methanol, 1 g. of powdered Girard T reagent and 1.3 cc. of acetic acid. With occasional shaking, the Girard reagent went into solution in about 20 min. After the solution had stood for another 1.5 hr. the solution was poured into 600 cc. of ice-water containing 5 cc. of 2 *N* sodium hydroxide solution and the Erlenmeyer flask was rinsed with two 20-cc. portions of ether. The mixture was then transferred to a separatory funnel, about 100 cc. more ether was added, together with 25 cc. of saturated sodium chloride solution (to aid in layer separation), shaken well and let settle. The aqueous layer was separated, washed with two 300-cc. portions of ether, and set aside. The combined ether layer and washings were washed with water, shaken with saturated sodium chloride solution, filtered through magnesium sulfate, and evaporated. The residual solid was boiled with 200 cc. of methanol, the volume reduced to 110 cc., and the solution was filtered and seeded while hot with a crystal of ketone 104. After 5 hr. the crystallizate that had separated was collected (2.62 g., m.p. 119–121°) and dissolved in the least methanol under reflux (about 100 cc.). After standing overnight the needles that separated were collected: 2.2 g. of ketone 104, m.p. 121.5–123°. The mother liquor was evaporated to dryness and the residue chromatographed; three crystallizations of the solid fractions from methanol afforded 400 mg. more of ketone 104, m.p. 120–122°.

Hydrolysis of the aqueous solution of Girard derivative gave 240 g. of ketonic residue that afforded 232 mg. of cholestanone semicarbazone, equivalent to 202 mg. of cholestanone, or 0.07% cholestanol in the commercial cholesterol.

Infrared Characterization of Ketone 104.—The principal bands in the spectrum taken on a Baird instrument are reported in paper III. Spectra in carbon bisulfide (Fig. 1) and in chloroform were kindly measured on a Perkin-Elmer instrument by Mrs. Phyllis B. Smeltzer at the National Institutes of Health through the courtesy of Dr. Erich Mosettig. In both solvents there is strong carbonyl absorption at 5.79 μ (1727 cm.$^{-1}$) and a weak shoulder at 5.94 μ (1684 cm.$^{-1}$). In both solvents strong bands appear at 5.78, 8.98, 9.81, 10.05 and 11.07 μ (1735, 1114, 995, 704 cm.$^{-1}$).

Derivatives of Ketone 104. (a) **Oxime.**—A mixture of 56 mg. of the ketone, 56 mg. of hydroxylamine hydrochloride, 3 cc. of methanol and 0.1 cc. of pyridine was refluxed for 3 hr. and the solution was concentrated and let cool, when 52 mg. of oxime crystallized as prismatic needles, m.p. 191–193°. Recrystallization from dilute methanol raised the m.p. to 194.6–195.2°.

Anal. Calcd. for $C_{27}H_{45}O_3N$ (431.64): C, 75.12; H, 10.50. Found: C, 75.03; H, 10.47.

(b) **2,4-Dinitrophenylhydrazone.**—A boiling solution of 50 mg. of ketone and 25 mg. of 2,4-dinitrophenylhydrazine in 10 cc. of methanol was treated with one drop of 36% hydrochloric acid, boiled for 5 min., and let cool. The crystalline hydrazone that separated was dissolved in benzene and the yellow solution passed through a column of 1.5 g. of alumina. The product was recovered by elution with benzene and crystallized twice from methanol-ether, which gave yellow silky needles, m.p. 182–182.4°.

Anal. Calcd. for $C_{33}H_{48}O_6N_4$ (596.75): C, 66.41; H, 8.11; N, 9.39. Found: C, 66.49; H, 8.11; N, 9.32.

(c) **Ethylenethioketal.**—Dry hydrogen chloride was passed into a solution of 105 mg. of ketone 104 in ethanedithiol kept at −15° for 15 min. The solution was let stand at 0° for 3 hr. and then solid sodium carbonate was added and the mixture extracted with ether. The ethereal solution was washed three times with 2 *N* sodium hydroxide and with water, dried and evaporated. On chromatography of the residual oil, petroleum ether eluted a solid product that on crystallization twice from methanol-acetone gave 47 mg. (38%) of elongated prisms, m.p. 103–104°, α_D −23.4° Chf (*c* 1.62), λ^{Chf} 8.98, 9.81, 10.05, 11.05 μ.

Anal. Calcd. for $C_{29}H_{48}O_2S_2$ (492.67): C, 70.68; H, 9.81; S, 12.99. Found: C, 70.66; H, 10.02; S, 12.76.

The yield was raised to 62% in a second experiment conducted by passing hydrogen chloride into a solution of 253 mg. of ketone and 2.5 cc. of ethanedithiol in 3 cc. of chloroform at 8–10° for one-half hr. and letting the solution stand at room temperature for 16 hr.

(d) **Desoxo Derivative.**—The above thioketal (200 mg.) was refluxed in absolute ethanol (50 cc.) with excess Raney nickel (about 1 g.) for 5 hr.; acetone (10 cc.) was added and refluxing was continued for a further 2 hr. The filtered solution was evaporated to dryness and the residue taken up in ether. The solution was washed, dried, and evaporated and the oily residue chromatographed. Elution with petroleum ether gave a total of 160 mg. of solid product, m.p. 54–57°, and this on three crystallizations from methanol–acetone gave material melting at 54.8–55.8°, α_D +2.9° Chf (*c* 1.62), λ^{Chf} 8.98, 9.5, (10.28), (11.07) μ.

Anal. Calcd. for $C_{27}H_{46}O_2$ (402.64): C, 80.54; H, 11.52. Found: C, 80.65; H, 11.54.

(e) **Alcohol 104 Acetate.**—A solution prepared by refluxing 1 g. of lithium aluminum hydride with 50 cc. of ether and filtering was added to a solution of 115 mg. of ketone 104 in 25 cc. of ether and the solution was refluxed for 1 hr. and worked up in the usual way. The product was an oil and no solid material could be obtained on chromatography. The alcohol as prepared had α_D −19.7° Chf (*c* 1.93), close to that of material purified through the acetate (below) and gave only a faint trace of precipitate with digitonin. The alcohol was eluted slowly by 1:1 petroleum ether–benzene, comparable to cholesterol, and there was no evidence of inhomogeneity. Acetylation with pyridine (2 cc.)-acetic anhydride (4 cc.) for 36 hr. at 25° and chromatography afforded 94 mg. (74%) of acetate, m.p. 99–101°. Three crystallizations from methanol gave silken needles, m.p. 101–101.5°, α_D −32.6 Chf (*c* 1.85), λ^{Chf} 5.76, 8.95, 9.78, 10.05, 11.05.

Anal. Calcd. for $C_{29}H_{48}O_4$ (460.67): C, 75.61; 10.50. Found: C, 75.77; H, 10.64.

Saponification of 110 mg. of crystalline acetate by refluxing for 1.5 hr. with 0.17 g. of potassium hydroxide in 5 cc. of methanol gave oily alcohol that could not be caused to crystallize; deacetylation with lithium aluminum hydride gave the same result. The purified alcohol had α_D −22.5° Chf (*c* 1.33); a solution in ethanol gave no precipitate on treatment with an alcoholic solution of digitonin. Treatment with bromine in acetic acid–ether by the standard method resulted in no precipitation.

Oxidation of 106 mg. of the oily alcohol in benzene (1.06 cc.)-acetic acid (1.4 cc.) with 38 mg. of dichromate in 0.4 cc. of acetic acid at 5–7° for 8 hr. and then at 25° for 12 hr. furnished 83 mg. of ketone 104.

A mixture of 125 mg. of the acetate, 0.7 g. of Raney nickel (wet), 50 cc. of methanol and 5 cc. of ethyl acetate was heated under hydrogen at 90–95° for 2 hr. (final pressure 950 lb./sq. in.), and the oily residue recovered was acetylated; the product was unchanged acetate, m.p. 100–101°. Hydrogenation was attempted in acetic acid in the presence of Adams catalyst, but no hydrogen was absorbed in 4 hr. Attempted Clemmensen reduction of 200 mg. of acetate in 50 cc. of 95% ethanol with 15 g. of amalgamated zinc and 3 cc. of 36% hydrochloric acid (3 cc. more added every hr.) over a 5-hr. period resulted only in recovery (after acetylation) of unchanged acetate (130 mg., m.p. 99–100°).

(f) **Ketone 104 Enol Acetate.**—A mixture of 100 mg. of ketone, 1.5 cc. of acetic anhydride and 98 mg. of aluminum chloride was heated for 4 hr. in a flask surrounded by the vapor of boiling xylene. A violent reaction occurs at the beginning and is controlled by adjusting the rate of heating. The slightly reddish reaction mixture was decomposed with ice and extracted with ether and the extract was washed with water and bicarbonate solution, dried and evaporated. Chromatography afforded 66 mg. (60%) of crystals, m.p. 111–113°. Three crystallizations from methanol gave rectangular prisms, m.p. 115.6–116°, α_D +15.9° Chf (*c* 1.74), λ^{Chf} 5.86, 6.1, 9.88, 10.10, 11.1 μ.

Anal. Calcd. for $C_{29}H_{46}O_4$ (458.66): C, 75.94; H, 10.11. Found: C, 75.77; H, 10.10.

The enol acetate absorbs bromine rapidly in carbon tetrachloride solution. A solution of 50 mg. of the substance in 3 cc. of methanol containing one drop of 36% hydrochloric acid was heated on the steam-bath and let stand overnight. Characteristic needles of ketone 104 separated, m.p. and mixed m.p. 123–124°, α_D −36.2° Chf (*c* 1.20).

(g) **Lactone.**—A chloroform solution (2 cc.) containing 0.138 g. of perbenzoic acid was added to 155 mg. of ketone 104, which promptly dissolved. The solution was let stand at 5–7° for 24 hr. and then at 25° for 18 hr., treated with water and ether and the ethereal layer washed with water and with bicarbonate solution, dried and evaporated. The residual oil, after attempted crystallization had failed, was refluxed for 1 hr. with 5 cc. of methanol and 1 cc. of Claisen alkali. The solution was diluted with water, extracted with ether, and the ether back-washed with 2 *N* sodium hydroxide solution. The combined aqueous solutions were acidified with hydrochloric acid at 25° and the precipitated gum recovered by extraction with ether. The washed and dried extract on evaporation gave 121 mg. of residue; a solution of this in methanol was cooled in a salt–ice-bath, and on scratching the lactone separated as a solid. Three crystallizations from methanol gave 25 mg. of needles, m.p. 89–90°, α_D +8.9° Chf (*c* 1.48), λ 5.80, 8.8, 11.15 μ.

Anal. Calcd. for $C_{27}H_{44}O_4$ (432.62): C, 74.95; H, 10.25. Found: C, 74.87; H, 10.36.

In another experiment the aqueous alkaline solution was cooled to 0°, acidified with cold (0°) 2 *N* sulfuric acid, and the precipitated gum extracted rapidly with chilled ether. The ethereal solution was washed twice with ice-water and poured into an ethereal solution of diazomethane. The solution was dried and evaporated and the residue chromatographed. The only solid product was the lactone, m.p. and mixed m.p. 89–90°.

(h) **Resistance to Acid.**—A solution of 100 mg. of ketone 104 in 5.6 cc. of methanol and 0.3 cc. of 95% sulfuric acid was refluxed for 3 hr. Crystallization of the product from methanol gave 85 mg. of unchanged starting material. A mixture of 90 mg. of ketone, 22 cc. of methanol, and 1 cc. of 95% sulfuric acid was refluxed for 16 hr., but only unchanged ketone 104 was recovered (70 mg.).

(i) **Action of Hypobromite.**—Four cc. of a solution of 3 g. of bromine in 44.4 cc. of 4% sodium hydroxide was added to a cold solution of 100 mg. of ketone 104 in 30 cc. of dioxane and 3 cc. of pyridine and the solution was let stand 1 hr. at 7–9° and 48 hr. at 25°. No acidic product was formed.

(j) **Action of Bromine.**—Ketone 104 did not absorb bromine in carbon tetrachloride or in acetic acid, with or without addition of dry hydrogen bromide.

(k) **Action of Girard's Reagent T.**—A mixture of 88 mg. of ketone 104, 6 cc. of methanol, 73 mg. of reagent and 0.1 cc. of acetic acid was refluxed for one-half hour, cooled, diluted with water and extracted with ether. The washed and dried ethereal extract furnished 32 mg. of unchanged ketone and the rest was recovered from the hydrolyzed aqueous layer. In another experiment 60 mg. of ketone was treated similarly with reagent (70 mg.) in methanol (25 cc.)–acetic acid (0.1 cc.) at 25° for 2 hr.; 55 mg. of unreacted ketone 104 was recovered from the ether phase.

(l) **Action of Phosphorus Oxychloride.**—A mixture of 105 mg. of ketone 104, 0.4 cc. of phosphorus oxychloride and 1 cc. of pyridine was let stand at 25° for 48 hr.; 80 mg. of unchanged ketone was recovered.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Chromic Acid Oxidation of Epicholesteryl Acetate

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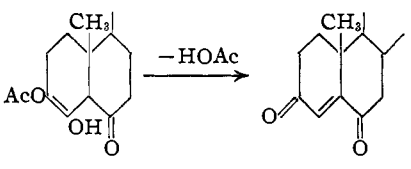
RECEIVED MAY 7, 1953

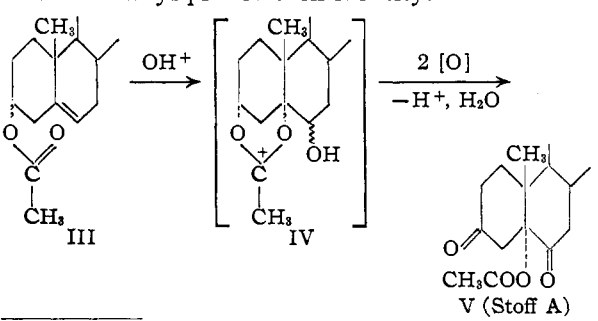
One of two products obtained by Windaus and Naggatz in 1939, by chromic acid oxidation of epicholesteryl acetate, has been identified as cholestane-3,6-dione-5α-ol 5-acetate (V). It is formed by oxidation in propionic acid as well as in acetic acid solution and hence results from an intramolecular $C_3 \rightarrow C_5$ acyl migration, probably involving a cyclic acetal (IV).

Windaus and Naggatz[2] investigated the oxidation of epicholesteryl acetate with chromic acid in acetic acid solution as the first step in the synthesis of 7-dehydroepicholesterol. They noted that the principal product, the expected 7-ketone, is distinguished from the 3β-acetoxy epimer by more ready elimination of acetic acid with formation of $\Delta^{3,5}$-cholestadiene-7-one; it is of interest that probably the 3α-acetoxy group is polar whereas the 3β-acetoxy group is equatorial. A second oxidation product isolated in 14% yield was designated "Stoff A" and shown to have the empirical formula $C_{29}H_{46}O_4$. It was characterized merely as being transparent to ultraviolet light and as convertible by chromatography on alumina or by treatment with alcoholic alkali into Δ^4-cholestene-3,6-dione (II), and the possible formulation I was suggested very tentatively and with evident lack of conviction.



I (Stoff A?) II

In a reinvestigation of this secondary oxidation product we were able to confirm all the observations of Windaus and Naggatz. The infrared spectrum provided further evidence of the presence of an acetate (5.76, 8.0 μ) and a carbonyl group (5.81 μ) but showed no band in the hydroxyl region. The only plausible way in which the elements of acetic acid can be considered to be added to Δ^4-cholestene-3,6-dione without producing a hydroxyl group is that leading to cholestane-3,6-dione-5α-ol-5-acetate (V). This substance was prepared by Hattori[3] by chromic acid oxidation of cholestane-3β,5α,6β-triol 5-acetate and the constants agree well with those of "Stoff A." Direct comparison of samples prepared in the two ways proved their identity.



III IV V (Stoff A)

(1) This work was conducted under a scholarship granted by the National Research Council of Canada.

(2) A. Windaus and J. Naggatz, *Ann.*, **542**, 204 (1939).

(3) J. Hattori, *J. Pharm. Soc. Japan*, **59**, 411 (1939); English abstract, *ibid.*, **59**, 129 (1939).