[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

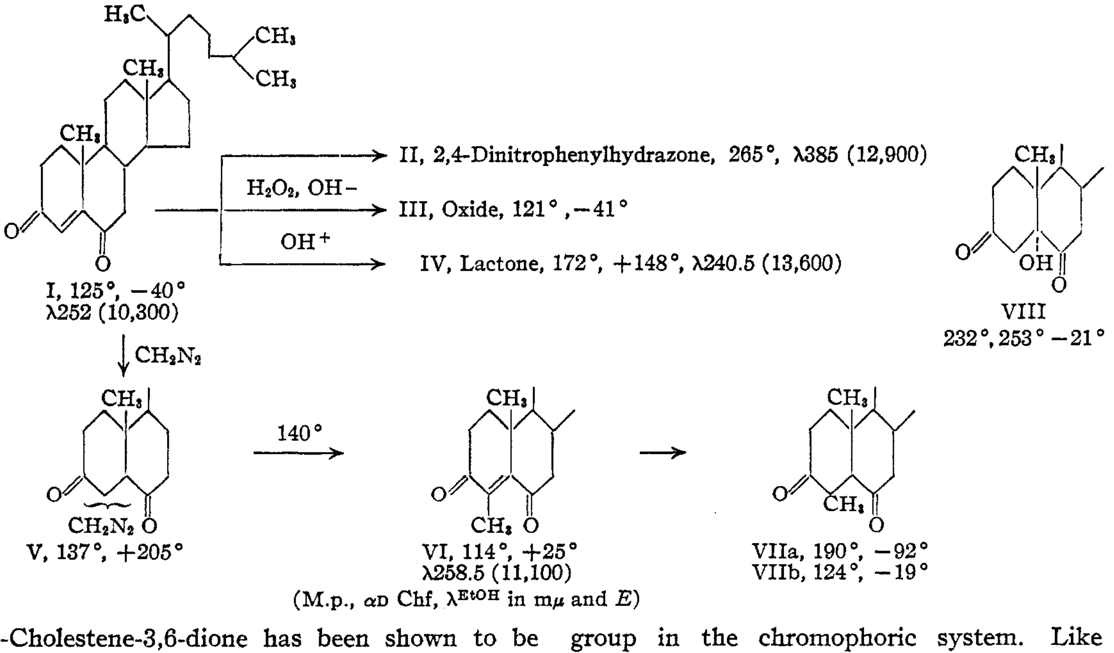
Cholesterol and Companions. II. Exhaustive Dichromate Oxidation

BY LOUIS F. FIESER

RECEIVED FEBRUARY 20, 1953

Oxidation of cholesterol with excess dichromate at a low temperature affords Δ^4-cholestene-3,6-dione as the chief product; exploratory experiments are reported on the reaction of this enedione with diazomethane, alkaline hydrogen peroxide and perphthalic acid. Cholestane-3,6-dione-5α-ol is a minor neutral oxidation product that can be isolated in yields of 2 and 4.8%, respectively, by oxidation with dichromate and with anhydrous chromic anhydride; it probably arises through the α- and β-oxides. The acid fraction resulting from dichromate oxidation of cholesterol contains, in addition to the Diels acid, an α,β-unsaturated keto diacid that Butenandt and Hausmann regarded as a 6-keto-Δ^4-2,3-secodioic acid. Reinvestigation has shown that the substance is a 3-keto-Δ^4-6,7-secodioic acid (XXII) and that the ketone resulting from refluxing the acid with zinc and acetic acid is B-norcoprostane-3,6-dione (XXVII).

When purified cholesterol is oxidized in acetic acid–benzene with excess dichromate (10 oxygen equivalents) the initially formed major product Δ^5-cholestene-3-one is oxidized first to Δ^4-cholestene-6β-ol-3-one (paper I) and then to Mauthner and Suida's[1] Δ^4-cholestene-3,6-dione (I). A small amount of cholestane-3,6-dione-5α-ol[1] is invariably present in the neutral fraction, and a considerable amount of the cholesterol is converted into acidic products, discussed below. The optimum conditions found for formation of Δ^4-cholestene-3,6-dione involve maintaining a temperature of 9–15° for 15–20 hr.; if the temperature is allowed to rise to 25° the enedione is almost all oxidized to acids in the same reaction period. Under the conditions specified the pure enedione can be isolated in 42% yield by a succession of crystallizations; a simpler preparative procedure is described in paper III (procedure C).



I, 125°, −40°
λ252 (10,300)

V, 137°, +205°

VI, 114°, +25°
λ258.5 (11,100)

VIIa, 190°, −92°
VIIb, 124°, −19°

VIII
232°, 253° −21°

(M.p., α_D Chf, λ^{EtOH} in mμ and E)

Δ^4-Cholestene-3,6-dione has been shown to be reducible with zinc dust to cholestane-3,6-dione,[2] to react very readily with phenylhydrazine to form a monophenylhydrazone,[1] to form a labile cyanohydrin,[3] an enol ethyl ether[4] and to react with hydrogen peroxide catalyzed by osmium tetroxide to give a 4,5-glycol.[5] The substance also forms a 2,4-dinitrophenylhydrazone (II), reacts with hydrogen peroxide in an alkaline medium to give an oxide (III), and with perphthalic acid to give a lactone (IV). In spite of the marked enolic properties, the enedione I does not give an enol ether on reaction with diazomethane but instead forms a pyrazoline derivative (V). This substance melts with evolution of nitrogen and affords a mixture, one component of which was isolated by chromatography. The properties of the product indicate that it is probably 4-methyl-Δ^4-cholestene-3,6-dione (VI), rather than the isomeric 4,5-methylenecholestane-3,6-dione or an A-homo enedione. The absorption maximum at 258.5 mμ seems to exclude the cyclopropane formulation but to be consistent with structure VI, since the displacement of the maximum from 252 mμ (I) to 258.5 mμ is about that expected for the substitution of an additional alkyl group in the chromophoric system. Like the parent enedione I, the pyrolysis product is reducible with zinc and acetic acid. Two isomeric dihydro derivatives are formed, which are probably the epimeric 4-methylcholestane-3,6-diones VIIa and VIIb; an A-homo enedione should form a single dihydride, as I does.

Mauthner and Suida's cholestane-3,6-dione-5α-ol

---

(1) J. Mauthner and W. Suida, *Monatsh.*, **17**, 579 (1896).

(2) A. Windaus, *Ber.*, **39**, 2249 (1906).

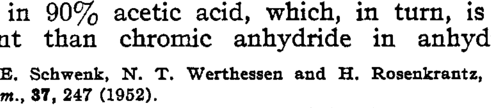
(3) van Oordt, Dissertation, Freiburg, 1901.

(4) A. Windaus, *Ber.*, **40**, 257 (1907).

(5) A. Butenandt and H. Wolz, *ibid.*, **71**, 1483 (1938).

(VIII) is easily isolated by virtue of its sparing solubility in alkane solvents and the yield is accurately determinable and reproducible. In the course of a search for companion substances, batches of cholesterol derived from a variety of tissues were purified through the dibromide and oxidized by a standard procedure and VIII was invariably isolated in yield of 1.9–2.1%. The substance is probably derived in part from cholestane-3β,5α,6β-triol present in the cholesterol. Schwenk and co-workers[6] isolated cholestane-3β,5α-diol-6-one from purified cholesterol and showed that the ketone can be formed by oxidation of cholestane-3β,5α,6β-triol by bromine under the conditions used for formation of the dibromide. Furthermore my associate Dr. Bidyut K. Bhattacharyya (paper VI) has isolated minute amounts of cholestane-3β,5α,6β-triol from cholesterol from gallstones, egg yolk and human brain, in the last case under conditions precluding its formation during the isolation. However, general experience in the chromatography of cholesterol of various sources practically precludes the possibility that these all contain as much as 2% of the triol. Evidence that the bulk of the material arises as a product of the oxidation of cholesterol is that when purified cholesterol from one of the same batches was oxidized by the anhydrous chromic anhydride–acetic acid procedure[7] the yield of cholestane-3,6-dione-5α-ol rose from 2 to 4.8%.

Mary Fieser and I[8] suggested that the 3,6-dione-5α-ol is formed *via* cholesterol α-oxide, from which it is indeed obtainable by oxidation with either chromic acid[9] or dichromate. We had expected that the β-oxide would give rise to the 3,6-dione-5β-ol, but it has now been found that cholesteryl acetate β-oxide (IX) on dichromate oxidation

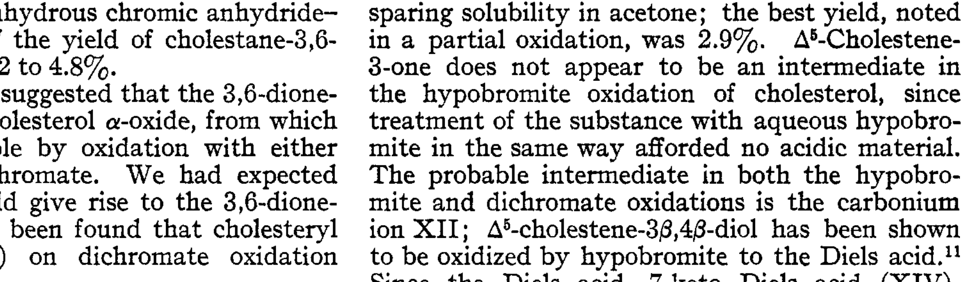


IX X

affords exclusively cholestane-3β,5α-diol-6-one 3-acetate (X). Hence the 3,6-dione-5α-ol may arise from either the α- or β-oxide of cholesterol.

Sodium dichromate in acetic acid or acetic acid–benzene is a slightly milder reagent than chromic acid in 90% acetic acid, which, in turn, is less potent than chromic anhydride in anhydrous acetic acid. In the experiments cited above chromic anhydride gave over twice as high a yield of a product probably derived from an oxidic intermediate as did dichromate. Sodium dichromate in acetic acid oxidizes anthracene and phenanthrene to the quinones less rapidly than does chromic acid but in about the same yields. Attempted dichromate oxidation of β-methylnaphthalene gave water-soluble products instead of the quinone. Oxidation of cholestanol and of 2,6-dimethylnaphthalene with anhydrous chromic anhydride gave cholestanone and 2,6-dimethyl-1,4-naphthoquinone in about the same yields as are obtained with sodium dichromate and chromic acid, respectively.

From the mixture of acids resulting from chromic acid oxidation of cholesterol, Windaus[2] isolated the Diels acid (XIII), identical with material obtained by Diels and Abderhalden[10] in 14% yield by oxidation of cholesterol suspended in aqueous sodium hypobromite. It was found in the present work that the acid can be isolated easily by virtue of its sparing solubility in acetone; the best yield, noted in a partial oxidation, was 2.9%. Δ^5-Cholestene-3-one does not appear to be an intermediate in the hypobromite oxidation of cholesterol, since treatment of the substance with aqueous hypobromite in the same way afforded no acidic material. The probable intermediate in both the hypobromite and dichromate oxidations is the carbonium ion XII; Δ^5-cholestene-3β,4β-diol has been shown to be oxidized by hypobromite to the Diels acid.[11] Since the Diels acid, 7-keto Diels acid (XIV), and dihydro Diels acid have not previously been fully characterized, optical constants are now reported.



XI XII XIII XIV

XIII: Dimethyl ester, m.p. 69°, αD −40.5°, λ216 (8390)

XIV: Dimethyl ester, m.p. 76°, αD −55.3°, λ230 (9240)

When the acid mixture from the acetone mother liquor remaining after separation of the Diels acid was esterified with diazomethane and the ester chromatographed, the sole crystalline product isolated proved to be isomeric with the diester of XIV but corresponded, as ester and as acid, with a substance obtained by Butenandt and Hausmann[12] in 4% yield by oxidation of Δ^4-cholestene-3,6-dione with chromic acid for 2 hr. at 70°. The Butenandt acid has now been obtained in yields up to 35% by oxidation with excess dichromate at 25° and purification either as the dimethyl ester or by crystallization of the free acid from ether–petroleum ether. Butenandt, without discussion, attributed formula XV to the acid and formula XVI to an interesting product resulting from brief refluxing

---

[6] E. Schwenk, N. T. Werthessen and H. Rosenkrantz, *Arch. Biochem.*, **37**, 247 (1952).

[7] L. F. Fieser, THIS JOURNAL, **70**, 3237 (1948); L. F. Fieser and J. Szmuszkovicz, *ibid.*, **70**, 3352 (1948).

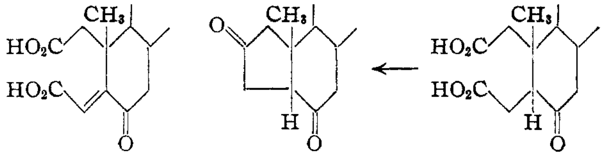
[8] L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Edition, Reinhold Publ. Corp., New York, N. Y., 1949, p. 230.

[9] T. Westphalen, *Ber.*, **48**, 1064 (1915); L. Ruzicka and W. Bosshard, *Helv. Chim. Acta*, **20**, 244 (1937).

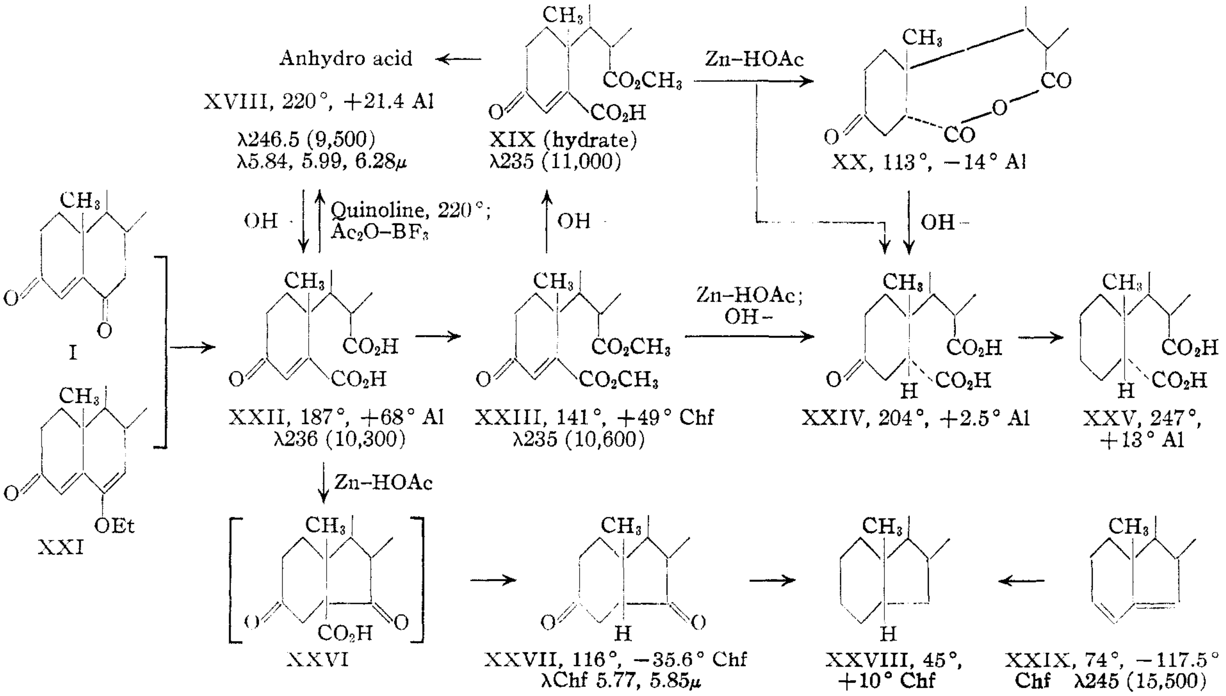
[10] O. Diels and E. Abderhalden, *Ber.*, **36**, 3177 (1903).

[11] O. Rosenheim and W. W. Starling, *J. Chem. Soc.*, 377 (1937).

[12] A. Butenandt and E. Hausmann, *Ber.*, **70**, 1154 (1937).


XV XVI XVII

with zinc dust and acetic acid. However, a substance of the structure XVI and differing from the Butenandt ketone was prepared by Windaus[13] by pyrolysis of the 6-keto-2,3-dioic acid (XVII) resulting from oxidation of cholestane-3,6-dione; the structure of XVII is unambiguous, since on Wolff–Kishner reduction it gave an acid different from the 6,7-dioic acid,[14] the only alternate possibility. The Butenandt ketone and XVI are listed in Elsevier[15] as stereoisomers, but it is doubtful if any but the A/B-*cis* form would have sufficient stability to exist; the Butenandt ketone, at least, is stable to alkali.



(Melting points, specific rotations, λ^{EtOH} in mμ and *E*)

The formulation XV for the acid also seemed questionable because it would imply enolization of Δ^4-cholestene-3,6-dione by shift of hydrogen from C_2 rather than from C_7. Evidence from ultraviolet absorption comparisons that the enol ethyl ether has the structure XXI is supported by chemical evidence to be presented in a later paper, and hence the observation that this substance on dichromate oxidation likewise yields the Butenandt acid affords strong evidence that the substance is 6,7 - seco - Δ^4 - cholestene - 3 - one - 6,7 - dioic acid[16] (XXII). The acid, as well as its dimethyl and monomethyl esters, has an absorption maximum close to that found for 7-keto Diels acid (XIV, 230 mμ). Reduction with zinc and acetic acid proceeded as reported and afforded the Butenandt ketone and carbon dioxide, both in yield of about 30%. A second reduction product not observed by Butenandt and Hausmann is an acid easily isolated as the water-insoluble disodium salt. The acid is ketonic and the analysis shows it to be dihydro Butenandt acid. Reduction of the dimethyl ester XXIII, followed by hydrolysis, gives dihydro Butenandt acid and none of the ketone. The saturated keto acid evolves no carbon dioxide when refluxed with zinc and acetic acid, but in a prolonged reaction period the carbonyl group suffers some reduction (to alcohols). Dihydro Butenandt acid (m.p. 204°) was converted both by Wolff–Kishner reduction and by desulfurization of the ethylene thioketal to a desoxo acid, m.p. 247°. If Butenandt's formulation XV were correct the dihydro acid should correspond to Windaus' 6-keto-2,3-dioic acid XVII (m.p. 219°) and the desoxo product to the corresponding 2,3-dioic acid[17] (m.p. 196°), but this is not the case. The keto and desoxo acids also differ from 6,7-seco-3-ketocholestane-6,7-dioic acid,[18] m.p. 255° (from 3α- and 3β-chlorocholestane-6-one) and 6,7-seco-cholestane-6,7-dioic acid,[19] m.p. 273° (from 6- and 7-ketocholestane). However, in this instance two stable stereoisomers are possible, and on the strength of supplementary evidence to be cited below it seems safe to conclude that the new acids are the secocoprostane derivatives XXIV and XXV. Although Δ^4-cholestene-3,6-dione is reduced to the 5α-dihydride, it is not particularly

---

[13] A. Windaus, *ibid.*, **36**, 3752 (1903).

[14] By nitric acid oxidation of 6- and 7-ketocholestane: A. Windaus, *ibid.*, **52**, 170 (1919); A. Windaus and O. Dalmer, *ibid.*, **52**, 162 (1919).

[15] "Elsevier's Encyclopedia of Organic Chemistry," Elsevier Publ. Corp., New York, N. Y., Vol. 14, 1940, p. 62.

[16] Ciba conference nomenclature, *J. Chem. Soc.*, 3526 (1951).

[17] A. Windaus, *Z. physiol. Chem.*, **117**, 146 (1921).

[18] A. Windaus and G. Stein, *Ber.*, **37**, 3699 (1904); A. Windaus and A. v. Staden, *ibid.*, **54**, 1059 (1921).

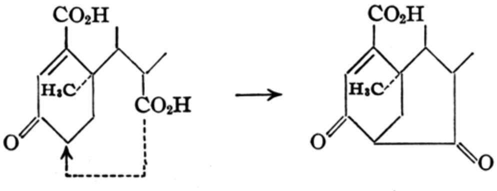
[19] A. Windaus and O. Dalmer, *ibid.*, **52**, 162 (1919); A. Windaus, *ibid.*, **53**, 488 (1920).

surprising that the Butenandt acid (XXII) affords the 5β-dihydride (XXIV).

Correlation with a known compound was effected by reduction of the Butenandt ketone XXVII to a hydrocarbon XXVIII both by the Huang–Minlon procedure and through the bis-ethylenethioketal. A comparison sample was obtained from $\Delta^{3,5}$-B-norcholestadiene XXIX, prepared according to Windaus and Resau[20] by pyrolysis of the crude acidic fraction resulting from chromic acid oxidation of cholesteryl acetate. I was unable to isolate a pure component of the acid mixture by crystallization or by chromatography of the ester, but pyrolysis of the mixture proceeded smoothly and afforded a sparingly soluble and nicely crystalline diene of sharp and constant melting point. The absorption maximum (245 mμ) is at a longer wave length than that of $\Delta^{3,5}$-cholestadiene (234 mμ), but the effect is probably attributable to the incorporation of one of the double bonds in a 5-membered ring; thus $\Delta^{8,14}$-cholestadienyl acetate[21] absorbs at 249 mμ (18,100) and not at 244 mμ, as expected from the usual relationships. Windaus[22] states that hydrogenation of XXIX in ether gave a mixture, m.p. 80°, but that hydrogenation in ether–acetic acid gave solely a hydrocarbon forming fine glistening plates, m.p. 45°. In the present work mixtures invariably were obtained, but fractionation of the more soluble material gave glistening thin plates melting at about 45°. The substance is difficult to purify and the melting point unsharp. However material of completely similar behavior resulted from reduction of the Butenandt ketone by both methods mentioned. The hydrocarbons from the two sources may contain impurities, but it appeared from the similarity in behavior and from mixed melting points and infrared spectra that they are substantially the same.

The preparation of Butenandt ketone (XXVII) from cholesterol with isolation of the intermediate enedione and Butenandt acid is tedious and the over-all yield is only 4.4%. Since the reductive cyclization reaction seemed likely to be unique to an acid of a specific structure, the following simplified procedure was tried and found very satisfactory: cholesterol was oxidized with excess dichromate at room temperature, a small neutral fraction was discarded, and the total acid mixture was refluxed with zinc and acetic acid. After removal of the acid fraction, the neutral fraction on crystallization afforded pure Butenandt ketone in 7.5% yield. In accord with the formulation, the infrared spectrum contains a carbonyl doublet indicative of the presence of both a 6-ring ketone (5.85 μ) and a 5-ring ketone (5.77 μ). The substance formed a mono-2,4-dinitrophenylhydrazone having a single band at 5.77 μ, and hence the selective condensation occurs at the 3-position. The observation already mentioned that the Butenandt ketone is not isomerized by alkali indicated that the substance has the more stable 5β-orientation, as in XXVII.

The possibility that conversion of the Butenandt acid to the ketone XXVII involves decarboxylation followed by cyclization was explored by heating the diacid with copper chromite in quinoline at 220°. No carbon dioxide was evolved, but the diacid ($C_{27}H_{42}O_5$) was transformed into an anhydro acid ($C_{27}H_{40}O_4$), listed as XVIII. The same substance was obtained in better yield by heating XXII in quinoline without catalyst and also by warming the diacid with acetic anhydride and boron fluoride etherate. The anhydro acid is saponified by very dilute alcoholic alkali in the cold with regeneration of XXII. The infrared spectrum of the anhydro compound indicates the presence of a free carboxyl group and an α,β-unsaturated keto group, and the ultraviolet absorption band is shifted from 236 to 246.5 mμ. The substance appears definitely to be ketonic, for it forms a water-soluble Girard derivative from which it can be regenerated. Dr. Robert Stevenson has suggested that the substance may be the β-diketone XXX. The formulation appears consistent with the properties noted, and inspection of a model (Fig. 1) shows the tricyclic A/B system to be completely free from strain. The ready "saponification" would, in fact, be a β-diketone fission. To be sure, the formation of such a product from XXII would represent a reaction of novel type.



XXII XXX (for XVIII)

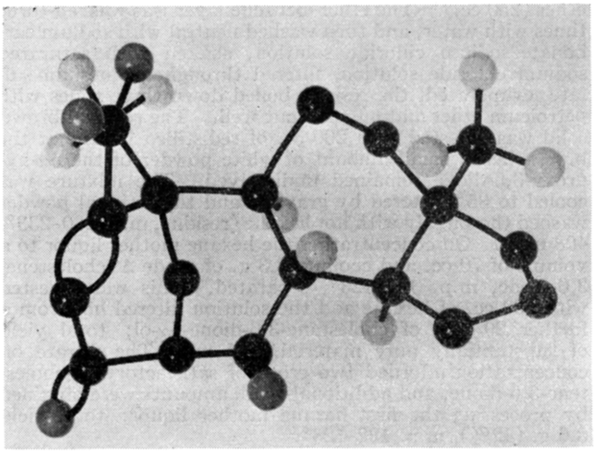


Fig. 1.—Model of formula XXX for anhydro Butenandt acid.

Another possibility investigated for clarification of the ketonization reaction was that a monomethyl ester of the Butenandt acid might afford an isolable ester of an intermediate product. Saponification of the diester XXIII in the cold afforded in high yield a monomethyl ester isolated as a nicely crystalline but indefinitely melting hydrate. This ester was converted by quinoline at 220° into the anhydro acid XVIII with elimination of methanol. Reduc-

---

(20) A. Windaus and C. Resau, *Ber.*, **48**, 851 (1915).

(21) W. J. Adams, V. Petrow and R. Royer, *J. Chem. Soc.*, 678 (1951).

(22) A. Windaus, *Ber.*, **53**, 488 (1920).

tion of the monoester with zinc and acetic acid afforded a mixture of dihydro Butenandt acid (XXIV), the anhydro acid XVIII, and a low-melting neutral substance characterized as the corresponding anhydride XX by saponification to the diacid XXIV. Since the diacid itself does not suffer dehydration under comparable conditions, the conversion to XVIII and to XX can be attributed to elimination of methanol in two directions. That neither the Butenandt ketone nor an intermediate ester was formed means that the carboxyl group eliminated in the ketonization reaction is that which, in the monomethyl ester, is esterified. The formation of the Butenandt ketone (XXVII) is evidently coupled to a process of reduction and does not involve pyrolysis of a reduction product. Perhaps the process is initiated by an attack by zinc at the keto group, followed by cyclodehydration to XXVI and decarboxylation. The monomethyl derivative is formulated as the 7-ester (XIX) because of the failure of the substance to undergo reductive ketonization.

Experimental

Oxidation Procedure B: Δ^4-Cholestene-3,6-dione (I) and Cholestane-3,6-dione-5α-ol (VIII).—Sodium dichromate dihydrate (51.2 g., 10 oxygen equiv.) was dissolved in 200 cc. of acetic acid by heating and the solution cooled to 15°. A solution of 20 g. of dibromide-purified cholesterol in 200 cc. of benzene was cooled to 10°, 200 cc. of acetic acid was added, the temperature was adjusted to 15°, and the cooled dichromate solution was added without delay (to avoid esterification of cholesterol). An orange precipitate separated at once to form a thick paste; this is identified as cholesteryl chromate by the fact that in the absence of cholesterol there is no precipitation. The flask was placed in a refrigerator (9°) and let stand overnight without further attention except to observe the temperature: 19° in 1 hr. (the cholesteryl chromate had largely dissolved); 17° in 2 hr., 15° in 3 hr., 9° in 17 hr. The clear, deep brown solution was diluted with water (750 cc.) and extracted with ether (250 cc.). The ether–benzene layer was washed three times with water, and then washed neutral with sodium carbonate–sodium chloride solution, shaken with saturated sodium chloride solution, filtered through magnesium sulfate, evaporated, the residue boiled down three times with petroleum ether and pumped out well. The residual brown solid was digested with 200 cc. of redistilled hexane at the b.p., when a small amount of white powder of the 5α-hydroxy-3,6-dione remained undissolved. The mixture was cooled to 35°, filtered by gravity, and the residual powder washed thoroughly with hot hexane (residue, m.p. 230–233°, 408 mg.). On concentrating the hexane mother liquor to a volume of 70 cc. and cooling, 8.3 g. of crude Δ^4-cholestene-3,6-dione, m.p. 122–123°, separated. This was digested with 100 cc. of hexane and the solution filtered hot from a further 29 mg. of cholestane-3,6-dione-5α-ol; total yield of substantially pure material, 437 mg. The filtrate on concentration afforded two crops of satisfactory Δ^4-cholestene-3,6-dione, and additional small amounts were obtained by processing the first hexane mother liquor; total yield 8.6 g. (42%), m.p. 122–123°.

As a method of preparing Δ^4-cholestene-3,6-dione, this procedure has the disadvantage that the separation from cholestane-3,6-dione-5α-ol takes time and may be incomplete and that the process at one point requires handling of 1.6 l. of liquids. Procedure C (paper III), although designed for another purpose, is better adapted to the preparation of Δ^4-cholestene-3,6-dione because, although the yield is lower, a product free from cholestane-3,6-dione-5α-ol is obtainable by a very simple work-up requiring the handling of only 0.8 l. of solvent mixture.

Properties of Δ^4-Cholestene-3,6-dione.—The best samples of the enedione, purified by crystallization, extraction with Claisen alkali, or by chromatography (eluted after cholestane and Δ^4-cholestenone and before cholesterol) formed light yellow plates from methanol, m.p. 124–125°, α_D −40° Chf (*c* 2.43), −23° Di (*c* 2.19), λ^{EtOH} 252 mμ (10,300).

Anal. Calcd. for $C_{27}H_{42}O_2$ (398.61): C, 81.35; H, 10.62. Found: C, 81.42; H, 10.63.

Oxide (III).—A suspension prepared by dissolving 4 g. of Δ^4-cholestene-3,6-dione in 150 cc. of 95% ethanol and cooling to 25° was treated with 4 cc. of Claisen alkali, which produced a yellow solution of the enolate, and then with 4 cc. of 30% hydrogen peroxide, added slowly with cooling. The yellow color soon was largely discharged and a sludge of sodium salt separated, largely on the walls of the flask. This precipitate was removed by two filtrations through cotton; the solution was then diluted and the mixture extracted with ether. Extraction with soda removed considerable acidic material, but this was not investigated. Evaporation of the ethereal solution gave neutral material that dissolved readily in petroleum ether and crystallized readily from a small volume of this solvent in padlike clusters of microneedles; yield in two crops, 0.46 g. and 0.18 g., m.p. 117–118°. Recrystallized, the oxide melted at 120–121°, α_D −41.0° Chf (*c* 2.37), λ^{Chf} 5.85 μ.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21. Found: C, 78.57; H, 10.30.

Reaction with Perbenzoic Acid.—In a preliminary experiment Δ^4-cholestene-3,6-dione was treated with excess perbenzoic acid in chloroform at 9°. After three days the initial yellow color had faded but not disappeared. The reaction product, collected by ether extraction, was readily soluble in methanol and crystallized, after rather extensive dilution with water, in small needles, m.p. 170°; recrystallized, m.p. 171–172°, α_D +148° Chf (*c* 1.945), λ^{EtOH} 240.5 mμ (13,600), λ^{Chf} 3.1, 5.75, 6.02, 6.21 μ; no coloration with Claisen alkali.

Anal. Calcd. for $C_{27}H_{42}O_3$ (414.61): C, 78.21; H, 10.21. Found: C, 78.30; H, 10.26.

The compound has the properties of a lactone. A solution of the substance and an equal weight of sodium dichromate was let stand overnight and starting material was recovered (mixed m.p. 167–169°). In another experiment the enedione was treated with excess reagent for one week at 9° and then for four days at 25° without discharge of the yellow color. Considerable acidic material was produced. Extraction of a hexane solution of the small neutral fraction with Claisen alkali or even with 6.25% aqueous sodium hydroxide gave a yellow solution of an enolate. The nature of the reaction is under investigation by a student.

2,4-Dinitrophenylhydrazone (II).—A solution of 1 g. of Δ^4-cholestene-3,6-dione and 0.5 g. of 2,4-dinitrophenylhydrazine in 70 cc. of 95% ethanol remained clear when heated but on addition of 0.1 cc. of 36% hydrochloric acid at once gave a thick paste, which on filtration afforded 1.4 g. of orange powder. The substance is sparingly soluble in benzene or acetic acid and moderately soluble in hot dioxane. On two crystallizations from dioxane it formed small round red-orange dots of microcrystals, m.p. 263–265° dec., λ^{EtOH} 385 mμ (12,900).

Anal. Calcd. for $C_{33}H_{46}O_5N_4$ (578.73): C, 68.48; H, 8.01. Found: C, 68.37; H, 7.99.

Pyrazoline Derivative (V).—A solution of 3 g. of Δ^4-cholestene-3,6-dione in 50 cc. of ether was treated with 25 cc. of an ethereal solution of diazomethane derived from 2.5 g. of nitrosomethylurea. In 35 min. round tufts of small colorless needles began to separate. The crystals were collected after another half-hour and the mother liquor concentrated to give a succession of crops, m.p. 134–136°, 132–134°, 131–133°, 126–129°; total yield 2.2 g. The substance is too readily soluble in acetic acid for crystallization and it forms gels from hexane. It is moderately soluble in methanol and separated in a cottony mass of fine needles. On recrystallization from ethanol, it separated slowly, first at the surface, in rosettes of small needles, m.p. 135–137° dec., α^{23}_D +205° Chf (*c* 2.34), λ^{Chf} 2.80 (shoulder), 2.94 (NH), 5.87 (CO).

Anal. Calcd. for $C_{28}H_{44}O_2N_2$ (440.65): C, 76.31; H, 10.07. Found: C, 76.41; H, 9.98.

When the substance was heated a little above the melting point, gas was evolved briskly for a few minutes and then gassing ceased. The product, an oily mixture, was dissolved in methanol and the solution cooled and scratched over a period of several days, when about one-third of the total separated in solid form. On chromatography of the solid, a succession of fractions eluted by petroleum ether on crystallization from methanol afforded large prisms of m.p. ranging from 111–116°. Fractions 2–8 and 9–16 on recrystallization gave the same result: large sheafs of flat tablets, m.p. 114–115°. A sample for analysis was recrystallized rapidly from acetic acid–water to provide small crystals; m.p. 114–115°, α^{22}D +25° Chf (*c* 2.68), λ^{EtOH} 258.5 mμ (11,100), λ^{Chf} 5.91, 5.98 (intense) μ. The analysis corresponds to that of **4-methyl-Δ^4-cholestene-3,6-dione** (VI).

Anal. Calcd. for $C_{28}H_{44}O_2$ (412.63): C, 81.50; H, 10.75. Found: C, 81.25; H, 10.89.

The substance is unaffected by refluxing with hydrochloric acid in methanol, and a hexane solution gives no yellow color when shaken with Claisen alkali.

When 0.55 g. of the unsaturated dione was refluxed overnight with zinc dust and acetic acid and the filtered solution was diluted with water, a solid product separated that appears to be a mixture of two **4-methylcholestane-3,6-diones**; yield 0.50 g., m.p. 114–122°. Crystallization from hexane–petroleum ether gave 82 mg. of thin plates of **isomer VIIa**, m.p. 177–179°. Recrystallization raised the m.p. to 189–190°, α^{21}D −92.3° Chf (*c* 2.29), λ^{Chf} 5.85.

Anal. Calcd. for $C_{28}H_{46}O_2$ (414.65): C, 81.10; H, 11.18. Found: C, 80.92; H, 11.14.

On chromatography of the mother liquors 3:1 petroleum ether–benzene slowly eluted six fractions of solid that on crystallization from methanol (fairly readily soluble) afforded **isomer VIIb**, which separated in clusters of short needles, m.p. 123–124° (unchanged by recrystallization), α^{21}D −18.7 Chf (*c* 2.73), λ^{Chf} 5.87 μ.

Anal. Calcd. for $C_{28}H_{46}O_2$ (414.65): C, 81.10; H, 11.18. Found: C, 80.70; H, 11.07.

Cholestane-3,6-dione-5α-ol, VIII. (a) By Oxidation with Anhydrous Chromic Anhydride.—A solution of 20 g. of cholesterol (Wilson Co. lot No. 83052) in 200 cc. of benzene was stirred and cooled to 10°, 400 cc. of acetic acid was added and the solution adjusted to 15°, when 20.6 g. of chromic anhydride was added. With efficient cooling, a temperature of 15° was maintained during an exothermic period of about $^3/_4$ hr., after which no cooling was required. The brown solution was let stand overnight, when it was purplish. Processing as in procedure B afforded 1.05 g. (4.8%) of cholestane-3,6-dione-5α-ol, m.p. 238°, unchanged on crystallization from ethanol with a little added dioxane (needles).

(b) **From Cholesterol α-Oxide.**—On addition of an acetic acid solution of 100 mg. of dichromate to a solution of 100 mg. of α-oxide, m.p. 147–148°, in acetic acid at 25° the chromate separated and in 1–2 min. dissolved. In one-half hour cholestane-3,6-dione-5α-ol began to separate; yield 90 mg., m.p. 226°; from methanol, m.p. and mixed m.p. 235°.

(c) **From Cholestane-3β,5α-diol-6-one.**—On addition of 240 mg. of dichromate to a solution of 0.84 g. of the diolone in 10 cc. of acetic acid, cooled to 30°, the product soon separated to give a thick paste. Water was added, 0.80 g. of product was collected (m.p. 220°) and crystallized from 20 cc. of acetic acid (0.52 g., 232°) and then from 190 cc. of methanol.

(d) **Properties.**—Present observations confirm citations in the literature indicating that the point of decomposition of highly purified cholestane-3,6-dione-5α-ol may vary from 232[23–25] to 253°.[26] The preparations mentioned above melted in the range 230–238°; a typical one, m.p. 236°, had αD −15° Di (*c* 0.39), −21° Chf (*c* 0.52).

Anal. Calcd. for $C_{27}H_{44}O_3$ (416.62): C, 77.84; H, 10.65. Found: C, 77.70; H, 10.84.

Another preparation when crystallized from dioxane–methanol melted at 253° dec.; another one, crystallized from benzene–hexane and then very slowly from ethanol, formed large, prismatic needles, m.p. 248° dec.

Mauthner and Suida[1] noted that the substance is dehydrated by alcoholic alkali or acid. If a crystal is covered with Claisen alkali in the cold the yellow color of the enolate soon appears around the crystal.

Oxidation of Cholesteryl Acetate β-Oxide (IX).—A solution of 300 mg. of oxide (m.p. 109.5–111°, prepared by E. J. Tarlton) in acetic acid was treated at 25° with 150 mg. of dichromate in acetic acid and let stand overnight. A few long needles separated, which when recrystallized from methanol gave 50 mg. of material, m.p. 230–231°, recrystallized from methanol gave 50 mg. of material, m.p. 230–231°, recrystallized, m.p. 230–231°, αD −55.2° Chf (*c* 1.05). The constants agree with those reported for cholestane-3β,5α-diol-6-one 3-acetate[27] (X, αD −55.8 Chf), and a mixture with a known sample[28] showed no depression in m.p.

Cholestanone. (a) Dichromate Oxidation.—Two grams of cholestanol (crystallized from 30–60° ligroin, m.p. 143–144°, αD +23.8° Chf (*c* 2.48) and 0.76 g. of sodium dichromate dihydrate were covered with 20 cc. of acetic acid and heated for 10 min. on the steam-bath. The solution was let cool and the crystals collected and washed colorless with methanol; 1.34 g., m.p. 129–130°. The mother liquor and washings were diluted with water, warmed to effect coagulation, and 0.53 g. of greenish product, m.p. 125–126°, collected. Crystallization of this from acetic acid gave 0.39 g. of colorless ketone, m.p. 129–130.5°; total yield 86%.

(b) **Anhydrous Chromic Anhydride.**[29]—A mixture of 4.3 g. of cholestanol, 1 g. of chromic anhydride and 35 cc. of acetic acid was stirred in a test-tube with a thermometer. In five minutes the temperature had reached 40°, and the suspension was then warmed to 60° in 10 min., when the last particles had dissolved. Then 40 cc. of water was added and the precipitate collected and dried; 4.1 g., m.p. 128–129°. Crystallization from methanol gave 3.0 g., m.p. 129–130°, and 0.6 g., m.p. 127–129°; total yield (83%).

Oxidation of Aromatic Hydrocarbons.—Sodium dichromate oxidized anthracene and phenanthrene in acetic acid at 85° (phenanthrene required 8 hr.) to the quinones but seemed to have no advantage over other agents. β-Methylnaphthalene consumed the reagent but was converted into water-soluble products. 2,6-Dimethylnaphthalene (15.6 g.) was dissolved in 100 cc. of hexane, 50 cc. of benzene and 100 cc. of acetic acid and the solution was stirred and treated in 5 min. with a solution of 30 g. of chromic anhydride in 20 cc. each of water and acetic acid while checking the temperature to 60° by cooling in an ice-bath. In another 5 min. cooling was no longer required; in 1 hr. (30°) the mixture was poured into a separatory funnel and the dark lower layer separated and discarded. The upper hydrocarbon layer was washed with successive small portions of water until free of chromium and acid, dried and evaporated, and the residue crystallized from methanol; total yield of bright canary yellow 2,6-dimethyl-1,4-naphthoquinone, 9.7 g. (53%).

Acidic Oxidation Products. Diels Acid. (3,4-Seco-Δ^5-cholestene-3,4-dioic Acid, XIII).—Purified (dibromide) cholesterol (20 g.) was oxidized according to procedure B except that only 20.4 g. of dichromate was used (4 oxygen equiv.) and the reaction mixture was kept at 20° for just 1.5 hr. The total acidic fraction was a glass weighing 2.20 g., and a solution of this material in hot acetone on cooling deposited a crystal crust of 420 mg. (1.9%) of Diels acid, m.p. 285°. In another oxidation conducted at 25° for 6 hr. the yield of acid crystallized once from acetone was 0.64 g. (2.9%). The **monomethyl ester**, prepared by esterification with methanol and boron fluoride etherate and crystallized from methanol–water, melted at 124–125°. The ester is soluble in hot bicarbonate solution; cholic acid behaves similarly, cholanic acid does not dissolve.

Oxidation of Δ^5-cholestene-3β,4β-diol was conducted by suspending 1 g. of material in a solution of 1 g. of sodium dichromate dihydrate in 20 cc. of acetic acid and warming the mixture until solution was complete (green). The acidic fraction on crystallization from acetone gave 42 mg. of Diels acid, identified as the monomethyl ester, plates from ligroin, m.p. and mixed m.p. 124.5–125.5°. The neutral fraction was tested with phenylhydrazine[1] for the presence of Δ^4-cholestene-3,6-dione with negative results.

---

[23] R. H. Pickard and J. Yates, *J. Chem. Soc.*, **73**, 1678 (1908).

[24] J. Mauthner and H. Suida, *Monatsh.*, **17**, 579 (1896).

[25] V. Prelog and E. Tagmann, *Helv. Chim. Acta*, **27**, 1867 (1944).

[26] A. Windaus, *Ber.*, **40**, 257 (1907).

[27] Fr. Schenck, *Z. physiol. Chem.*, **243**, 119 (1936).

[28] L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **71**, 3938 (1949).

[29] G. Vavon and B. Jakubowicz, *Bull. soc. chim.*, [4] **53**, 581 (1933), state that it is advantageous to conduct the oxidation "in acetic acid containing no water," but they evidently dissolved the chromic anhydride in water in the conventional manner.

Optical constants have not previously been reported. Determinations of ultraviolet spectra were kindly done at Birkbeck College through the courtesy of Dr. D. H. R. Barton. Diels acid had λ^{EtOH} 215 mμ (9,500); the dimethyl ester, m.p. 68–69°, had λ^{EtOH} 216 mμ (8,390), α^{22}D −40.5° Chf (*c* 2.16). Dihydro Diels acid dimethyl ester, m.p. 124–126°, had α^{22}D −10° Chf (*c* 1.51); literature,[30] −9° An.

7-Keto Diels Acid. 3,4-Seco-Δ^5-cholestene-7-one-3,4-dioic Acid Dimethyl Ester.—The acid was prepared according to Windaus[31] by permanganate oxidation of Diels acid. Analyses reported by both Windaus and Butenandt for acid purified by crystallization are 0.5–0.8% high for carbon, and Windaus' analysis for the monomethyl ester is 2% low for carbon. Consequently the crude acid was esterified with diazomethane and the diester chromatographed. Petroleum ether eluted oils and then 4:1 petroleum ether–benzene eluted material that solidified when rubbed with methanol. The ester is very soluble in methanol and when the cold solution is diluted with water it separates as an emulsion that slowly changes to well-formed stout needles, m.p. 75–76°, α^{21}D −55.3° Chf (*c* 3.00), λ^{EtOH} 230 mμ (9,240), λ^{Chf} 5.80, 5.96, 6.20 μ.

Anal. Calcd. for $C_{29}H_{46}O_5$ (490.66): C, 73.38; H, 9.77. Found: C, 73.35; H, 9.92.

Butenandt Acid.[12] **6,7-Seco-Δ^4-cholestene-3-one-6,7-dioic Acid** (**XXII**).—A solution of 12 g. of crude Δ^4-cholestene-3,6-dione in 100 cc. of acetic acid was mixed at 25° with a solution of 16 g. of dichromate in 100 cc. of acetic acid. The temperature rose to 27° in 1 hr. and 28° in 3 hr. After 3 days the greenish brown solution was diluted with water and extracted with ether. The ether extract was washed three times with a large volume of water, when considerable acetone-soluble tar separated on the walls of the funnel. Suction filtration of the wet solution through a pad of Norit removed all the tar and gave a pale greenish filtrate. This was extracted with bicarbonate solution and the extract was processed as described below. Evaporation of the solution containing the neutral fraction gave a small amount of solid that on crystallization from benzene–hexane afforded cholestane-5α-ol-3,6-dione, m.p. 237–238°. The bicarbonate extract was acidified and the oily acid extracted with ether. The dried ethereal solution was concentrated to a volume of about 10 cc., diluted with 40–50 cc. of petroleum ether, and boiled down to the point of crystallization. The first crystallizate melted at 173–176°, but on recrystallization from ether–petroleum ether in the same way afforded 0.87 g. of pure Butenandt acid, m.p. 185–187°, λ^{EtOH} 236 mμ (10,300), λ^{Chf} 5.89, 5.97, 6.23 μ (also carboxylic band).

The mother liquor material from the crystallization on esterification with diazomethane and crystallization from methanol gave 0.60 g. of dimethyl ester, m.p. 138–140°. The first diacid mother liquor deposited a second crop of sticky acid that afforded 1.3 g. of satisfactory, if greenish, dimethyl ester, m.p. 138.5–139.5°; total yield 20.5%.

A better yield resulted from partial oxidation with a large excess of reagent for a shorter period of time. Thus solutions at 25° of 14 g. of pure Δ^4-cholestene-3,6-dione in 120 cc. of acetic acid and of 28 g. of dichromate in 175 cc. of acetic acid were mixed and the mixture let stand for 17 hr. Processing as above afforded a neutral fraction that on crystallization from methanol gave 3.3 g. of starting material, m.p. 123.5–124.5°. The acidic fraction was treated with excess diazomethane and the resulting dimethyl ester crystallized from methanol; yield 2.5 g., m.p. 135–136°, 1.9 g., m.p. 136–137°; total yield based on material consumed, 35%.

Dimethyl Ester.[12] (**a**) **From Cholesterol.**—Oxidation of 5 g. of purified cholesterol in 100 cc. of benzene and 200 cc. of acetic acid (cooled to 5°) with 7.7 g. of dichromate (6 oxygen equiv.) in 50 cc. of acetic acid and 25 cc. of benzene at 3–9° for 24 hr. gave 1.7 g. of acidic fraction, and a solution of this in acetone deposited 0.15 g. of Diels acid, monomethyl ester m.p. 124–125°. The mother liquor material was esterified with diazomethane and the ester chromatographed.

Petroleum ether–benzene (4:1 and 1:1) eluted three oily fractions and then five crystalline fractions that on slow evaporation of solutions in ether gave clusters of large prismatic needles. The combined crops on recrystallization from methanol gave a total of 227 mg. of dimethyl ester, m.p. 139–140°. On recrystallization the substance separated very slowly in needles, m.p. 140–141.5°, αD +49° Chf (*c* 1.36), λ^{EtOH} 235 mμ (10,600), λ^{Chf} 5.83, 5.98, 6.22 μ, m.p. undepressed on admixture with the material described above.

Anal. Calcd. for $C_{29}H_{46}O_5$ (474.66): C, 73.38; H, 9.77. Found: C, 73.32; H, 9.80.

(b) **From Δ^4-Cholestene-3,6-dione Enol Ethyl Ether** (**XXI**).—Oxidation of 200 mg. of enol ether (m.p. 163–164°, prepared by Koji Nakanishi) with 200 mg. of dichromate in 20 cc. of acetic acid proceeded rapidly to a brown stage at 25°. After 15 hr. the mixture was processed as usual and the acidic fraction esterified with diazomethane. Crystallization from methanol gave 50 mg. of diester, m.p. 129–132°. Three further crystallizations raised the m.p. to 138–139°, mixed m.p. with (a) 139–140°.

Monomethyl Ester (**XIX**).—A solution of 2.6 g. of pure diester in 70 cc. of 95% ethanol was cooled to 25°, treated with 1.4 cc. of 25% sodium hydroxide solution, let stand for 30 min., diluted with water, and acidified. The monoester separated initially as an emulsion but this soon changed to a paste of well-formed needles, m.p. 122–126° (with gassing); yield 2.4 g. (92%). A batch of somewhat greenish dimethyl ester (1.9 g.) was saponified in the same way but after dilution with water the solution was filtered through a pad of Norit, which effectively removed the color; the yield of colorless needles, m.p. 120–132° dec., was 1.2 g. The substance is readily soluble in methanol, sparingly so in petroleum ether, too soluble in hexane for good crystallization. It crystallizes nicely from methanol–water in clusters of large needles of material that appears from the analysis to be a hydrate. The point of decomposition is not sharp but usually in the range 115–130°. A sample was crystallized twice by dissolving it in ether, adding 2–3 volumes of petroleum ether, and boiling the solution down to a point near saturation. The ester separated very slowly in one large round cluster made up of pointed blades, m.p. 122–125°; the second analysis reported was done on this material and shows it to be the hydrate.

Anal. Calcd. for $C_{28}H_{44}O_5 \cdot H_2O$ (478.65): C, 70.26; H, 9.69; H_2O, 3.77. Found: C, 70.53, 70.43; H, 9.63, 9.75; H_2O (loss in weight at 135°), 4.04.

The constants found for the pure hydrate are: αD +50° Chf (*c* 3.08), λ^{EtOH} 235 mμ (11,000), λ^{Chf} COOH band, 5.82, 5.97, 6.22 μ. The substance dissolves only sparingly in boiling bicarbonate solution.

Anhydro Butenandt Acid (**XVIII**). **Possible Structure, XXX.**—This substance was obtained first by heating 480 mg. of Butenandt acid with 50 mg. of copper chromite catalyst in 4 cc. of quinoline at 210–220° for 20 min. The cooled mixture was taken up in ether and the solution filtered, washed free of quinoline with hydrochloric acid and filtered through a pad of Norit by suction (without drying). The acidic product was then extracted with sodium carbonate solution and recovered from the aqueous layer by acidification and extraction with ether. The dried solution was then diluted with three volumes of petroleum ether and evaporated until crystallization started, when the substance separated as a white crystal powder or as fibrous needles; yield 170 mg., m.p. 219°. Recrystallization gave material of constant m.p. 219–220°, αD +21.4° Al (*c* 1.78), λ^{EtOH} 246.5 μ (9,500), λ^{Chf} COOH band, 5.84, 5.99, 6.28 μ.

Anal. Calcd. for $C_{27}H_{40}O_4$ (428.59): C, 75.66; H, 9.41. Found: C, 75.58; H, 9.43.

By a process conducted similarly but without addition of decarboxylation catalyst, 100 mg. of Butenandt acid yielded 58 mg. of anhydro acid, m.p. and mixed m.p. 220–221°. Material of the same m.p. and mixed m.p was also obtained by heating the Butenandt acid in acetic anhydride and boron fluoride etherate for 2 hr. on the steam-bath. The solution became quite brown, but crystallization as above gave colorless fibrous needles. The same product (m.p. 217–219°, mixed m.p. 218–219°) also resulted on heating the monomethyl ester with copper chromite in quinoline at 220° for 20 min.; the solution was swept with nitrogen and the gas passed into barium hydroxide, but no carbon dioxide was detected. The anhydro acid also resulted from heating the monomethyl ester in quinoline alone.

The anhydro acid was not altered by being refluxed in acetic acid for 2 hr. Saponification of XVIII and crystallization of the product from ether–petroleum ether gave the

---

(30) v. Burckhard and T. Reichstein, *Helv. Chim. Acta*, **25**, 1434 (1942).

(31) A. Windaus, *Ber.*, **41**, 611 (1908).

Butenandt acid, m.p. and mixed m.p. 183–185°. The anhydro acid dissolves readily in aqueous bicarbonate solution and it forms a water-soluble Girard derivative from which it can be regenerated. No crystalline ketonic derivatives could be isolated.

Dihydro Butenandt Acid. 6,7-Secocoprostane-3-one-6,7-dioic Acid (XXIV).—Butenandt acid dimethyl ester (480 mg.) was refluxed with a large excess of zinc dust in acetic acid overnight and the solution diluted with water and extracted with ether. The ethereal extract was washed with water and with bicarbonate solution, dried and evaporated. The residue was an oil that did not crystallize on brief processing (or after re-esterification with diazomethane), but when it was warmed in 10 cc. of methanol with 2 cc. of 25% aqueous sodium hydroxide solution a sodium salt soon started to precipitate to give a thick paste. This salt proved to be very sparingly soluble in water, but readily soluble in methanol, and so 25 cc. of water was added and the suspension was heated on the steam-bath for 15 min. to drive off the bulk of the methanol. The salt was then collected on a suction funnel, washed with water and dissolved in a little methanol. The solution was acidified, diluted with water and the acid was recovered by extraction with ether. When the dried ethereal solution was diluted extensively with petroleum ether and slowly evaporated, the saturated diacid separated as a microcrystalline powder, m.p. 200–201.5°; yield 100 mg. Recrystallization raised the m.p. to 203–204°; α^{23}_{D} +2.5° EtOH (*c* 1.61), λ^{Chf} carboxyl band, 5.83, 5.96 μ.

Anal. Calcd. for $C_{27}H_{44}O_5$ (448.62): C, 72.28; H, 9.89. Found: C, 72.12; H, 9.71.

The **dimethyl ester**, prepared with diazomethane, crystallized from methanol extensively diluted with water as small needles, m.p. 107–108°; recrystallized, m.p. 108–109°.

Anal. Calcd. for $C_{29}H_{48}O_5$ (476.67): C, 73.07; H, 10.15. Found: C, 72.76; H, 10.20.

Ethylenethioketal of XXIV.—Addition of ethanedithiol and boron fluoride etherate to a solution of dihydro Butenandt acid in acetic acid at 25° caused prompt separation of the ketal derivative in a thick paste. The diacid, washed with methanol and dried, melted with decomposition at temperatures ranging from 278 to 295°. It formed gels from benzene but crystallized fairly well from acetic acid on long standing in soft tufts of micro crystals, m.p. about 280° dec.

Anal. Calcd. for $C_{29}H_{48}O_4S_2$ (524.67): C, 66.38; H, 9.22. Found: C, 66.58, 66.50; H, 9.33, 9.47.

The **dimethyl ester** (diazomethane) was crystallized by dissolving it in methanol (long boiling) and evaporating the filtered solution to a small volume. The ester separated as small crystal aggregates, m.p. 142.5–143.5°; recrystallized, m.p. 144–145°.

Anal. Calcd. for $C_{31}H_{52}O_4S_2$ (552.85): C, 67.33; H, 9.48. Found: C, 67.22, 67.24; H, 9.47, 9.59.

Desulfurization of the diacid with Raney nickel proved unsatisfactory, since the bulk of the product was retained by the metal; a very small amount of reduced material corresponded in m.p. (240°) to the product of Wolff–Kishner reduction described below.

6,7-Secocoprostane-6,7-dioic Acid (XXV).—A mixture of 1 g. of the sodium salt of dihydro Butenandt acid, 1 cc. of 85% hydrazine hydrate, 1.5 g. of potassium hydroxide and 20 cc. of diethylene glycol was heated until the frothing subsided, refluxed for 1 hr., evaporated to a temperature of 200° and refluxed for 2 hr. After dilution and acidification, the reduced acid was collected by ether extraction. Evaporation gave 0.75 g. of a white solid, m.p. 235° dec. This was dissolved in ether, 3 volumes of petroleum ether was added, and the solution evaporated to the point of saturation. The acid separated as microcrystals melting with decomposition, on successive crystallizations, at 243–245°, 246–248°, 246–247°, α^{22}_{D} +13° EtOH (*c* 1.53). A mixture with dihydro Diels acid (m.p. 257–258°) melted at 225°.

Anal. Calcd. for $C_{27}H_{46}O_4$ (434.64): C, 74.61; H, 10.67. Found: C, 74.75; H, 10.77.

Attempted conversion of the diacid to a pyroketone by slow distillation of a solution of the substance in acetic anhydride and then vacuum distillation of the residue was unsuccessful. The distillate was an oil and afforded an oily 2,4-dinitrophenylhydrazone that on chromatography gave no crystalline fractions. Pyrolysis of the barium salt also afforded no crystalline product.

The **dimethyl ester** (diazomethane) crystallized from a methanol–water mixture adjusted to a point where it would stay clear for some time at 7–9°. The twice crystallized ester formed a cottony mass of fine needles, m.p. 72–73°.

Anal. Calcd. for $C_{29}H_{50}O_4$ (462.69): C, 75.28; H, 10.89. Found: C, 75.17; H, 10.89.

Dihydro Butenandt Acid Anhydride (XX).—One gram of the Butenandt acid monomethyl ester was refluxed overnight with zinc dust and acetic acid and the solution was filtered, diluted with water and the product extracted with ether. The solution was washed three times with water and then extracted with two portions of bicarbonate solution, which removed a small amount of dihydro Butenandt acid; purification through the sodium salt and crystallization from ether–petroleum ether gave 56 mg., m.p. and mixed m.p. 203.5–204.5°. Extraction of the ether solution with sodium carbonate solution then removed a small amount of a second acid that appeared to be anhydro Butenandt acid; it crystallized from ether–petroleum ether in round clusters, m.p. 205°, mixed m.p. 210° (found: C, 74.86; H, 10.02). The true neutral fraction recovered from the ether solution on crystallization from methanol gave 270 mg. of prisms of the anhydride, m.p. 100–102°. Successive recrystallizations gave long, flat, slender blades of m.p. as follows: 108–110°, 109–112°, 112–113°, 112–113°, α^{23}_{D} −10° Chf (*c* 2.37), −14° Al (*c* 1.80), λ^{Chf} 5.78, 5.85, 7.88 μ; saturated to tetranitromethane.

Anal. Calcd. for $C_{27}H_{42}O_4$ (430.61): C, 75.31; H, 9.83. Found: C, 75.47; H, 10.01.

The crude product, m.p. 100–102°, may be contaminated with the Butenandt ketone, since saponification of another batch of crude neutral product afforded a small neutral fraction. The product of saponification was purified through the sodium salt and identified as dihydro Butenandt acid, m.p. 204–205°, mixed m.p. 203.5–205°, α^{23}_{D} +0.8° EtOH (found: C, 72.49; H, 9.79).

Butenandt Ketone. B-Norcoprostane-3,6-dione (XXVII). (A) **From Pure Acid.**—A mixture of 1.2 g. of Butenandt acid, 3 g. of zinc dust and 30 cc. of acetic acid was refluxed overnight and an ethereal extract of the diluted mixture washed well with water and extracted with bicarbonate. The extract deposited a salt that afforded 145 mg. of crude dihydro Butenandt acid (m.p. 180°; this yielded 128 mg. of pure ethylenethioketal derivative). The neutral material from the ethereal solution solidified readily when rubbed with methanol, and crystallization from methanol (very soluble) gave 250 mg. of needles, m.p. 115–116°, and 45 mg., m.p. 114.5–115.5°; yield 28%. The first crop material had α_{D} −35.6° Chf (*c* 2.81), λ^{Chf} 5.77, 5.85 μ.

Anal. Calcd. for $C_{26}H_{42}O_2$ (386.60): C, 80.77; H, 10.95. Found: C, 80.77; H, 10.98.

In another experiment 338 mg. of acid was refluxed with zinc and acetic acid under nitrogen for 1.5 hr. and the gas swept into barium hydroxide; the yield of barium carbonate was 48 mg. (32%). No further carbon dioxide was evolved on adding more zinc dust and refluxing further.

The ketone was recovered unchanged after being heated on the steam-bath with alcoholic alkali for 1 hr. A solution of 180 mg. of ketone and excess semicarbazide in absolute ethanol was heated for 1 hr. on the steam-bath and allowed to concentrate, but no crystals separated. Addition of water precipitated a granular tan solid, which was dried to constant weight (207 mg.) and crystallized from methanol; a small amount of tan powder of poor appearance separated, m.p. 197–200° dec. This and the material recovered from the mother liquor was heated with potassium hydroxide in diethyleneglycol at 200° for 3 hr., but nothing but black products of decomposition resulted. Cholestanone decomposed when similarly treated.

(b) **From Cholesterol.**—A solution of 150 g. of dichromate in 400 cc. of acetic acid was cooled to 10° and added to a solution at 20° of 40 g. of cholesterol in 400 cc. of benzene and 400 cc. of acetic acid. The mixture was kept at 15° for 1 hr. and then let stand for 20 hr. at room temperature. Water was added, the mixture extracted with ether, the ether–benzene layer washed three times with water and then extracted thoroughly with bicarbonate solution and discarded. The bicarbonate extract was back-washed with ether, then acidified and the acid mixture collected by ether extraction and obtained as a glass (23 g.). This material was refluxed for 2 hr. with 20 g. of zinc dust and 100 cc. of acetic acid and the solution was filtered, diluted with water and extracted with ether. The ethereal solution was washed well with water and with dilute hydrochloric acid and then extracted with bicarbonate solution. A white sodium salt separated in the aqueous phase and was collected by filtration. After further extraction with sodium carbonate solution the ethereal solution was dried and evaporated. The residual oil (4.7 g.) on being rubbed with methanol yielded a total of 3.0 g. (7.5%) of Butenandt ketone, m.p. 113–115°. The crude sodium salt (4.4 g.) afforded acid that did not crystallize well and so the sodium salt was reformed, suspended in boiling water, collected by filtration of the hot mixture and then shaken with dilute hydrochloric acid and ether. The dried ethereal solution was concentrated and the ether largely displaced by petroleum ether, when good crystals of dihydro Butenandt acid separated, m.p. 204–205°.

A second experiment was conducted in the same way except that oxidation was continued for 44 hr. and the period of refluxing with zinc and acetic acid extended to 17 hr. The second change apparently resulted in some reduction of both the Butenandt ketone and the dihydro Butenandt acid. The yield of the ketone obtained by crystallization was only 0.97 g. (m.p. 113–114°), and on chromatography of the mother liquor material considerable oily material was eluted by petroleum ether, and 3:1 petroleum ether–benzene eluted only 0.25 g. of Butenandt ketone, m.p. 114–115°. Only a small amount of sodium salt separated in the extraction with bicarbonate and it yielded only 80 mg. of dihydro Butenandt acid. However, the total acids from the water-soluble sodium salts on oxidation with dichromate afforded insoluble sodium salt that yielded 184 mg. of pure dihydro Butenandt acid, m.p. 204–205°. Thus the prolonged refluxing with zinc and acetic acid had effected some reduction of the keto group to hydroxyl.

(c) **2,4-Dinitrophenylhydrazone.**—A solution of 173 mg. of Butenandt ketone and 87 mg. of 2,4-dinitrophenylhydrazone in the least volume of hot 95% ethanol was treated with three drops of 36% hydrochloric acid, when the derivative promptly began to separate. After digestion on the steam-bath for one-half hr. the granular yellow product was collected. The substance is sparingly soluble in ethanol or acetic acid, moderately soluble in dioxane. It crystallized from dioxane–ethanol in a crust of yellow microcrystals, m.p. 255–258° dec. (213 mg.), λ^{Chf} 3.05, 5.77, 6.15, 6.25, 6.65 μ.

Anal. Calcd. for $C_{32}H_{46}O_5N_4$ (566.72): C, 67.81; H, 8.18. Found: C, 67.81; H, 8.08.

(d) **Bis-ethylenethioketal.**—Attempts to condense Butenandt ketone with ethanedithiol in acetic acid and methanol with catalysis by boron fluoride gave only oils. However, the reaction proceeded well in the absence of solvent. Thus 360 mg. of ketone was treated with 1 cc. of ethanedithiol and 1 cc. of boron fluoride etherate; the solid dissolved and in 6 min. crystals of the reaction product began to separate and soon formed a thick paste. This was thinned with methanol and the solid collected; yield 482 mg. (96%), m.p. 178–185°. Crystallization from dioxane–methanol gave stout, pointed needles, m.p. 199–200°, α_D −30.7° Chf (*c* 1.82).

Anal. Calcd. for $C_{30}H_{50}S_4$ (538.70): C, 66.88; H, 9.36. Found: C, 66.61; H, 9.39.

Desulfurization of this derivative with Raney nickel in absolute ethanol and crystallization of the product from ether–methanol gave thin plates of material, m.p. 41.5–42.5°, that appeared to be identical with the hydrocarbon described in the next section; a mixture melted at 38–40°, solidified promptly, and remelted at 41–42°.

(c) **Wolff-Kishner Reduction to B-Norcoprostane (XXVIII).**—Reduction of the Butenandt ketone by the Huang-Minlon procedure gave a brown oil that when chromatographed afforded in the early petroleum ether eluates a colorless oil that was obtained crystalline only with considerable difficulty from methanol–ether mixtures, from which it tended to oil out. Better results were obtained by crystallization from a fairly large volume of acetic acid; the hydrocarbon separated very slowly in thin plates, m.p. 44–45°, α_D +10° Chf (*c* 1.47).

Anal. Calcd. for $C_{26}H_{46}$ (358.63): C, 87.07; H, 12.93. Found: C, 87.07; H, 12.97.

A comparison sample was prepared according to Windaus and Resau.[30] Oxidation of cholesteryl acetate was conducted by a simplification of an earlier procedure.[32] A mixture of 77.2 g. of cholesterol and 200 cc. of acetic anhydride was refluxed for 1 hr., 700 cc. of acetic acid and 52 g. of sodium acetate were added, the mixture was stirred mechanically, the temperature adjusted to 56–58°, and 60 g. of chromic anhydride was added in 30 min. (occasional cooling required) at 56–58°. The same temperature was maintained, later by gentle heating, for a total of 4.5 hr. and the solution was then cooled to 25° and diluted with 120 cc. of water. The **7-ketocholesteryl acetate** that crystallized was collected and washed with water and then methanol until colorless; yield 30.6 g., m.p. 155–157°. The mother liquor was diluted extensively with water, extracted with ether, and the acids extracted with aqueous ammonia. The neutral fraction on crystallization from methanol (4.1 g., m.p. 145–147°) and then 80% acetic acid afforded 2.7 g. more of the 7-ketone, m.p. 156–157°. The acid mixture was processed in various ways including chromatography after esterification with diazomethane, but no solid product could be isolated. Pyrolysis of about half of the total crude acids as described by Windaus gave 3.2 g. of yellow semisolid. When chromatographed, this afforded a series of early fractions eluted by petroleum ether that solidified promptly. Crystallization from 95% ethanol (sparingly soluble) or better from a large volume of acetic acid (40 cc./g.) gave 1 g. of well formed needles of **$\Delta^{3,5}$-B-norcholestadiene** of constant m.p. 73.5–74.5°, α_D −117.5° Chf (*c* 2.05), λ^{EtOH} 245 mμ (15,500). Hydrogenations of this diene, conducted by Dr. Wei-Yuan Huang, invariably gave mixtures of high and low melting products. Lower melting material predominated on using palladium–charcoal catalyst and ether–acetic acid as solvent. Fractional crystallization from acetic acid afforded, in the more soluble fractions, material separating as thin plates melting in the range 44–48°. A sample melting at 44–45° to a not fully clear melt when mixed with the above Wolff-Kishner product melted in the same way at 43.5–45°; the infrared spectra of the two samples were indistinguishable.

CAMBRIDGE, MASS.

---

(32) L. F. Fieser, M. Fieser and R. N. Chakravarti, THIS JOURNAL, **71**, 2226 (1949).