20-dione 21-acetate. The analytical sample was crystallized from benzene, m.p. 222–229°, $[\alpha]_D$ +61°; $\lambda_{max}^{KBr}$ 2.93, 3.31, sh.5.75, 5.81, sh.5.87, sh.5.93, 7.90, 8.10 $\mu$. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.15; H, 9.00. Chromatography of the mother liquors on 7.0 g. of acid-washed alumina (Merck) and elution with chloroform–ether 3:7 and 4:6 afforded additional anhydro product (13 mg.). Chloroform–ether 6:4 and 7:3 eluted 60 mg. of crystalline product, m.p. 190–200°. Rechromatography of the latter fraction on 5.0 g. of acid-washed alumina and elution with chloroform–ether 4:6 afforded 26 mg. of 5-methylpregnane-11$\beta$,17$\alpha$,21-triol-3,20-dione 21-acetate, m.p. 205–211°, $[\alpha]_D$ +78°, after three crystallizations from benzene; $\lambda_{max}^{KBr}$ 2.92, sh.5.75, 5.81–5.86, sh.5.90, 7.88, 8.08 $\mu$. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 67.76; H, 8.49.

**5-Methyl-1-pregnene-11$\beta$,17$\alpha$,21-triol-3,20-dione Acetate (IX).**—The cleavage of the bismethylenedioxy protecting group and acetylation of 200 mg. of VI was carried out as described above. The crude product was chromatographed on 17 g. of acid-washed alumina. Elution with ether afforded 22 mg. of recovered starting material. Elution with chloroform–ether 7:3 to chloroform afforded 101 mg. of material, m.p. 180–215°, which on rechromatography and then two crystallizations from benzene yielded the analytical sample of 5-methyl-1-pregnene-11$\beta$,17$\alpha$,21-triol-3,20-dione acetate, m.p. 209–215°, $[\alpha]_D$ +140°; $\lambda_{max}^{KBr}$ 2.93, sh.5.73, 5.80, 6.02, sh.6.17, 7.91, 8.13 $\mu$; $\lambda_{max}^{methanol}$ 233 m$\mu$, $E$ 6,900. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 69.10; H, 8.39.

RAHWAY, N. J.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY, HARVARD UNIVERSITY]

# Origin of Ketone 104 and Isolation of a Companion Acid[1]

BY LOUIS F. FIESER, WEI-YUAN HUANG[2a] AND TOSHIO GOTO[2b]

RECEIVED AUGUST 14, 1959

Ketone 104 is shown to be derived from cholesterol and not a companion, and analysis of the infrared and nuclear magnetic resonance spectra indicate that it is a spiroketal or spiroacetal. Synthetic 4,5-secocholestane-(3$\alpha$,5$\alpha$)(3$\beta$,4)-dioxide (**5**) is not identical with desoxoketone 104 but is similar in showing very strong infrared bands in the fingerprint region. Dichromate oxidation of cholesterol at 100° destroys other neutral products and affords ketone 104 in 4% yield. Oxidation at 121° leads to the easily isolated duoannelic acid (**8**). Another oxidation procedure affords crystalline Butenandt acid (**7**) in 12% yield.

Ketone 104, one of several products resulting on oxidation of commercial cholesterol with sodium dichromate in benzene–acetic acid,[3] has previously been characterized as a somewhat hindered ketone of the formula $C_{27}H_{44}O_3$ probably containing two oxidic bridges.[3,4] Because of the low yield (about 1%) and the high oxygen content, we at first thought it possible that the substance arises from an oxygen-rich companion rather than from cholesterol itself. However, a search for such a precursor proved fruitless[5] and we can now present evidence that cholesterol is the actual precursor. In view of later developments the experiments will not be recorded in detail but are summarized as follows. First a procedure was developed for oxidation of a 300–400-mg. sample, chromatography of the neutral fraction, removal of cholestanone (from cholestanol, present as a companion) and $\Delta^4$-cholestene-3,6-dione by Girard separation (usually repeated), conversion of ketone 104 to the 2,4-dinitrophenylhydrazone, and determination of the yield by spectrophotometry based upon the following constants: derivative, $E_{342}$ = 17,500, $E_{357.5}$ = 21,400; reagent: $E_{342}$ = 14,400, $E_{357.5}$ = 10,800. Typical results for oxidation of two samples of cholesterol purified through the dibromide and of unpurified commercial cholesterol were: yield of ketone 104: 1.19, 1.12 and 1.16%. Then $\Delta^4$-cholestene-3,6-dione was purified by the highly selective process of extraction from ligroin with Claisen alkali as the enolate and transformed into $\Delta^5$-cholestene-3$\beta$-ol. This sample on oxidation by the analytical procedure afforded ketone 104 in the same yield as obtained from other samples. In further confirmation of the point, we now report oxidation of methyl 3$\beta$-hydroxy-$\Delta^5$-cholenate to a dioxidic ketone fully analogous to ketone 104.

As previously reported,[4] the infrared spectrum of ketone 104 is characterized by four extremely strong bands in the fingerprint region. Drs. M. E. Wall and C. R. Eddy kindly examined the infrared region and noted a significant analogy to the sapogenins. Whereas ordinary steroids show absorption bands in the fingerprint region of molar absorptivities seldom exceeding 30 liters per mole per centimeter,[6] sapogenins are exceptional in having several sharp bands in this region with molar absorptivities[7] up to 422 l./mole$^{-1}$/cm.$^{-1}$. These bands are attributable to the spiroketal system in rings E and F, since opening of this ring system is attended with marked decrease in absorption in the fingerprint region.[7]

CHARACTERISTIC BANDS OF KETONE 104[a]

| Wave length, $\mu$ | Wave no., cm.$^{-1}$ | Molar absorptivity, l./mole$^{-1}$/cm.$^{-1}$ |
|---|---|---|
| 11.08 | 904 | 445 |
| 10.02 | 998 | 565 |
| 9.81 | 1019 | 604 |
| 8.97 | 1114 | 572 |

[a] Measurements by Dr. Eddy.

The oxidation product is clearly not a spiroketal of the sapogenin type, since it is very resistant to

(1) The Editors kindly consented to a trial in this and a few papers to follow of having formula numbers set in boldface arabic type as a means of obviating the many disadvantages of roman numerals.—L. F. FIESER.

(2) (a) Work done as postdoctoral fellow in 1954–1955; (b) Recipient of a Fulbright travel grant on leave from Nagoya University, Nagoya, Japan.

(3) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953).

(4) L. F. Fieser and B. K. Bhattacharyya, *ibid.*, **75**, 4418 (1953).

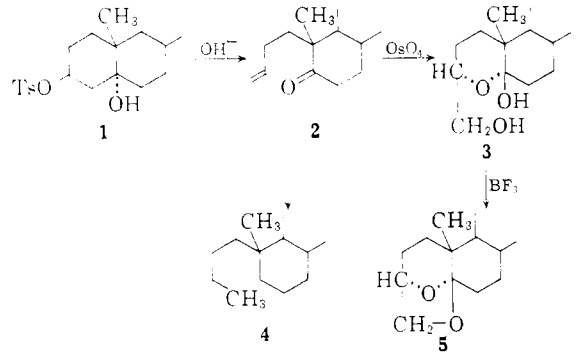
(5) L. F. Fieser, W.-Y. Huang and B. K. Bhattacharyya, *J. Org. Chem.*, **22**, 1380 (1957).

(6) R. N. Jones, E. Katzenellenbogen and K. Dobriner, THIS JOURNAL, **75**, 4418 (1953).

(7) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

acid hydrolysis, but the strong bands would appear to be indicative of the presence of a spiroketal or spiroacetal system of some other type.

After B. K. Bhattacharyya had spent the bulk of the year 1954–1955 investigating the degradation of ketone 104 with results which we only recently have been able to interpret (see two later papers), one of us (W.-Y. H.) sought an approach to the problem by the synthesis of a substance now regarded as 4,5-secocholestane-(3$\alpha$,5$\alpha$)(4,5$\beta$)-dioxide (**5**),




since this represented one possible structure for the desoxo derivative[4] of ketone 104. Clayton, Henbest and Smith[8] found that cholestane-3$\beta$,5$\alpha$-diol 3-tosylate (**1**) on reaction with potassium *t*-butoxide affords chiefly cholestane-3$\alpha$,5$\alpha$-oxide but gives also about 25% of 4,5-seco-$\Delta^3$-cholestene-5-one (**2**). In repeating this work we chromatographed the reaction mixture on acid-washed alumina and isolated epicholesterol, evidently arising by cleavage of the oxide by the adsorbent. The seco-enone **2** on reaction with osmium tetroxide gave an oily product which showed no carbonyl band but which had a very strong hydroxyl band in the infrared and which is probably a mixture of the ketol **3** and stereoisomers. Treatment with boron fluoride etherate at room temperature closed the second oxide bridge to give the ketal 5, of infrared spectrum devoid of hydroxyl or carbonyl bands but showing very strong bands at 8.75, 9.55, 9.80, and 11.13 $\mu$ analogous to those characteristic of ketone 104 and its desoxo derivative. The constants of the synthetic compounds (m.p. 102°, $\alpha_D$ +20° Chf), however, showed the substance to be an isomer of desoxoketone 104 (m.p. 55°, $\alpha_D$ +2.9° Chf.). That the product of synthesis shows highly distinctive fingerprint absorption similar to that of the oxidation product indicated that the latter probably has a dioxidic ring system similar to that of **5**.

At a later date nuclear magnetic resonance spectra kindly determined and interpreted by Dr. James N. Shoolery provided further guidance. From a study of the n.m.r. spectra of ketone 104 and its desoxo derivative, Dr. Shoolery concluded that these compounds have three protons with resonances shifted away from the main body of protons by the presence of adjacent oxygen atoms. Two large peaks in the spectra of both ketone 104 and its desoxo derivative at 95 and 99 cycles per second and two smaller flanking peaks present a pattern characteristic of two structurally non-equivalent protons attached to the same carbon atom. The shift value of about 95 c.p.s. is characteristic of a $CH_2$ group adjacent to an oxygen atom in a ring, and the sharpness of the lines strongly suggests that there are no protons adjacent to this methylene group. A peak at 35 c.p.s. is assigned to a proton attached to a carbon atom directly bonded to two oxygen atoms. The synthetic dioxide **5** does not conform to the requirements outlined. The n.m.r. data also eliminated certain other structures which otherwise had seemed worthy of consideration but still left a choice between various possibilities and did not lead to a unique solution of the structural problem.

The configuration attributed in formula **5** to the synthetic dioxide is that of estimated greater conformational stability and is supported by analogy to evidence to be presented later. The n.m.r. spectrum determined by T. Goto is reported in Fig. 1 with his interpretation. This and other spectra were obtained at 40 megacycles/sec. in $CDCl_3$; the resonance position of external benzene furnished the zero of reference. Bands that can be identified are lettered, and the assignments are given in the legend.

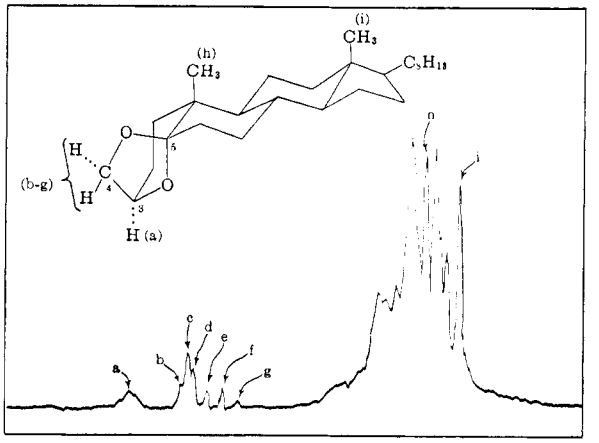



Fig. 1.—N.m.r. spectrum of 4,5-secocholestane-(3$\alpha$,5$\alpha$)-(4,5$\beta$)-dioxide in cycles per sec.: a, 73 (3$\alpha$-H); b, 97; c, 100; d, 103; e, 109; f, 116; and g, 123 (4$\alpha$-H and 4$\beta$-H); h, 213 (10-$CH_3$); i, 229 (13-$CH_3$).

**Oxidation Procedures.**—Further study of the preparation of ketone 104 led to results of interest. Previous procedures[3,4] have involved dichromate oxidation of cholesterol in benzene–acetic acid at a low temperature, separation of a neutral fraction, removal from this of $\Delta^4$-cholestene-3,6-dione by extraction from benzene–petroleum ether with Claisen alkali, removal of cholestanone by selective conversion to the Girard derivative, and chromatographic purification of ketone 104. The idea for an improved procedure came from a test in which solutions of the three neutral products in acetic acid containing dichromate were heated together on the steam-bath, from which it was evident that ketone 104 is very much more stable to oxidation than the two companion substances. The results reported in Table I of a series of comparisons conducted more recently demonstrate the extraordinary stability of ketone 104 to oxidation. The initial ob-

(8) R. B. Clayton, H. B. Henbest and M. Smith, *J. Chem. Soc.*, 1982 (1957).

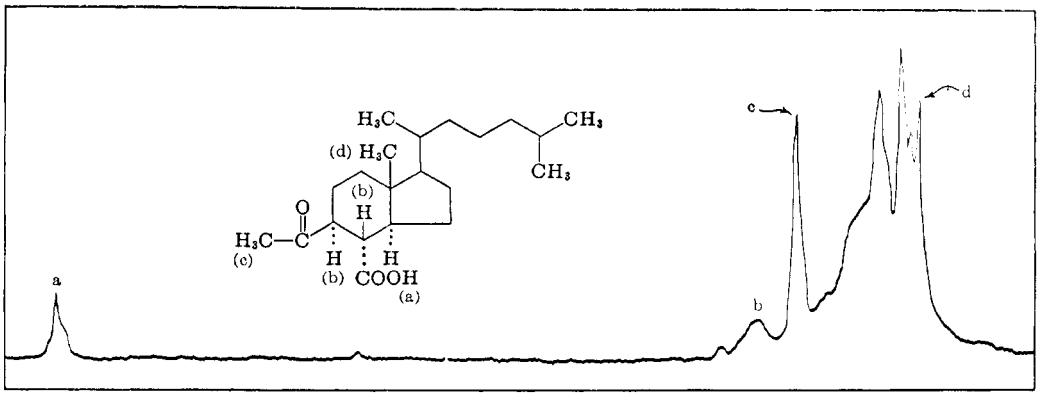



Fig. 2.—N.m.r. spectrum of duoannelic acid in cycles per sec.: a, −157 (carboxyl proton); b, 147 (8β-H, 9α-H); c, 168 (methyl ketone); d, 229 (13-$CH_3$).

servation suggested that the isolation of ketone 104 might be simplified by oxidizing cholesterol under conditions sufficiently drastic to destroy the other two components of the neutral fraction. Undergraduate Michael J. Chamberlin developed a procedure in which a hot (70°) solution of 150 g. of dichromate in acetic acid was added to a boiling solution of 20 g. of cholesterol in a little benzene, the initially vigorous exothermic reaction was allowed to proceed with distillation of some of the solvent, more solvent was removed by deliberate distillation, and the mixture was refluxed for 4 hr. Direct crystallization of the neutral fraction was reported to afford pure ketone 104 in amount slightly higher than the 1% yield previously obtained. The report did not record the amount of solvent removed and was ambiguous with respect to the temperature during the reflux period. In a first check experiment the initial exothermic reaction was let run under reflux, solvent was removed (benzene, water, acetic acid) until the vapor temperature reached 110° (liquid temperature 121°) and the solution was refluxed for 4 hr. Crystallization of the neutral fraction afforded pure ketone 104 in yield of 1.2%. On the supposition that this unusual oxidation product although remarkably stable to dichromate oxidation is not completely indestructible, further runs were made in which no solvent was removed and a reflux temperature of 100° was maintained. After reflux periods of 4, 2 and 1 hr., the yields of ketone 104, obtained pure by one crystallization of the neutral fraction from methanol, were 3.4, 3.9 and 4.0%. There seemed little possibility that the very substantial increase in yield could be due merely to the increased ease of isolation of ketone 104 when the other neutral products had been oxidized to acids, but the point was tested experimentally. Cholesterol (20 g.) was oxidized initially at a temperature of 18–42° (20 hr.) with 75 g. of dichromate, and then a second 75-g. portion of dichromate was added and the mixture refluxed for 1 hr. Ketone 104 was isolated easily by direct crystallization, but the yield was only 1.3%. A high temperature of oxidation thus does favor the abnormal reaction leading to ketone 104, and the procedure cited, with a 1-hr. reflux period, makes the unusual product very readily accessible.

TABLE I

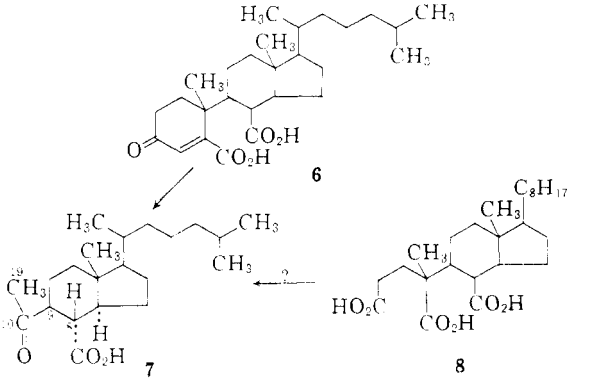
STABILITY TO 10 ML. OF A 1% SOLUTION OF $Na_2Cr_2O_7 \cdot 2H_2O$ IN ACETIC ACID AT 100°

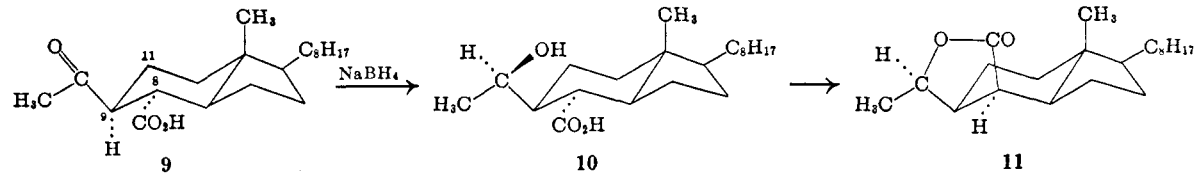
| Sample (100 mg.) | Brown | Yellow-green | Pure green |
|---|---|---|---|
| $\Delta^4$-Cholestene-3,6-dione | | | 5 min. |
| Butenandt acid | 7 min. | 24 min. | 1.5 hr. |
| Cholestane-3-one | 8 min. | 24 min. | 2 hr. |
| $\Delta^4$-Cholestene-3-one | 8 min. | 1 hr. | 3 hr. |
| 7-Ketocholesteryl acetate | 45 min. | 3.5 hr. | 10 hr. |
| Ketone 104 | 1 hr. | 16 hr.[a] | |

[a] Only faintly green; after dilution, the solution on cooling deposited 61 mg. of needles of unchanged ketone, m.p. 123–124°.

An initial experiment in which the reaction mixture was refluxed at a temperature of about 121° for 4 hr. afforded, in addition to 187 mg. of ketone 104 (from 20 g. of cholesterol), 1.7 g. of an acidic fraction which largely crystallized from petroleum ether. Repeated recrystallization to remove a persistent contaminant afforded an acid, m.p. 145°, of the formula $C_{21}H_{36}O_3$. The infrared spectrum of the methyl ester indicated that the substance is a keto acid, and separation through the Girard derivative was then found to simplify isolation of the pure acid. Thus a procedure was developed whereby the pure acid is obtained easily in 3.9% yield.

The n.m.r. spectrum of the new keto acid (Fig. 2) contains a sharp peak (c) at 168 c.p.s. attributable to the three protons of a methyl ketone; another peak at 229 c.p.s. is characteristic of the $C_{13}$-angular methyl group, and the usual band at 213–220 c.p.s. for a second angular methyl group is missing.






9 10 11

The spectrum of the methyl ester is similar but the peak (a) is replaced by an ester methyl peak at 110 c.p.s. The spectrum, analysis and source of the acid suggested the formula **7**. The acid was later obtained by drastic oxidation of the Butenandt acid, **6**, and of a mixture of nonketonic acids possibly containing Windaus' triacid **8**.[9] The carbon atoms of the methyl and carbonyl group thus correspond to original atoms $C_{19}$ and $C_{10}$. In analogy with Wieland's solannelic acid[10] in which ring D alone is intact, we name the new substance duoannelic acid (it is of simpler structure than **8**, which might be called duoannelic triacid).

Supporting evidence is that the substance on reduction with sodium borohydride gave a crystalline dihydroduoannelic acid which on acid-catalyzed dehydration afforded a $\gamma$-lactone (5.62 $\mu$). Since the reduction is highly stereospecific, it seems safe to infer the configuration of the product by application of the Prelog–Cram rule.[11] If in the model or conformational formula **9** the carbonyl group is flanked by the small group (9$\alpha$-H) and the medium group (11-$CH_2$) and points away from the large group (8-$CHCO_2H$), and if hydride ion attacks on the least hindered side of the small group, the configuration of the alcohol is that shown in **10** (hydrogen to the rear, hydroxyl to the front). The carbinol and carboxyl groups must be *trans*, and indeed brief refluxing of the hydroxy acid with methanolic hydrochloric acid effected only esterification. Conversion to the lactone required refluxing for 12 hr. and evidently was attended with inversion at $C_8$ to produce the 8$\beta$,9$\beta$-lactone **11**.

Brief attempts to trace the sequence of oxidative steps leading to duoannelic acid were not successful. A serious difficulty is that the polybasic acids involved have properties unfavorable for isolation. Hypoiodite oxidation of duoannelic acid gave a crystalline diacid (duoannelic diacid), but this was not encountered as a product of dichromate oxidation. Windaus isolated the triacid **8** as the crystalline monopotassium salt but was unable to crystallize acid regenerated from the pure salt. The Butenandt acid (**6**) was previously obtained crystalline only by oxidation of cholesterol to $\Delta^4$-cholestene-3-one (40% yield[12]) and further oxidation of this substance (20% yield[13]). We have now found that the production of crystallizable material is greatly facilitated by separation of ketonic from nonketonic acids with Girard reagent and also by ether extraction of a bicarbonate solution of the acid, for this effectively removes a sludgy contaminant. A new procedure for low-temperature oxidation and further processing makes crystalline Butenandt acid available directly from cholesterol in 12% yield. Drastic oxidation of both the Butenandt acid and the non-ketonic acids accompanying it afforded ketonic and non-ketonic acids, and in each case the former fraction yielded duoannelic acid.

It is interesting that the two substances which withstand extremely drastic conditions of dichromate oxidation, ketone 104 and duoannelic acid, both retain the intact sterol side chain. The preferred procedures for side chain degradation call for the addition of considerable amounts of sulfuric acid, and the best yields are obtained by oxidation at 0°.[14]

**Acknowledgments.**—This investigation was supported by grants from the National Cancer Institute of the National Institutes of Health (CY-1696, Endo), Research Corporation, and the Higgins Fund of Harvard University. We are indebted to Dr. J. N. Shoolery for n.m.r. data and interpretation.

## Synthetic Experiments (W.-Y. H.)

**4,5-Seco-$\Delta^3$-cholestene-5-one**[15] (**2**).—A solution of 4.8 g. of cholestane-3$\beta$,5$\alpha$-diol[16] (m.p. 217–218°) and 6 g. of *p*-toluenesulfonyl chloride in 40 ml. of pyridine was let stand at room temperature for 17 hr. and diluted with ice-water. A crystalline solid separated and on crystallization from a large volume of methanol gave a first crop of 4.75 g. of the 3-tosylate, 137–138°, dec., $\alpha_D$ −3°, and a second crop m.p. 135–137° dec. The basic reagent was prepared by dissolving 1 g. of either sodium or potassium in 100 ml. of *t*-butyl alcohol; 2.4 g. of the diol 3-tosylate was added and the solution was refluxed for 2 hr. Most of the solvent was removed at reduced pressure and the residue was diluted and extracted with ether and the ethereal solution was washed free of alkali and the recovered product chromatographed on 40 g. of acid-washed alumina. Petroleum ether–benzene (4:1 to 1:1) eluates afforded about 0.6 g. of 4,5-seco-$\Delta^3$-cholestene-5-one; $\lambda^{Chf}$ 5.85, 6.05, 10.95 $\mu$; 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 145–150°. Benzene–ether eluates (19:1 to 1:1) gave 0.6 g. of epicholesterol (crystallized from chloroform–methanol: m.p. 140–142°, $\alpha_D$ −41° Chf.).

**4,5-Secocholestane-(3$\alpha$,5$\alpha$)(4,5$\beta$)-dioxide** (**5**).—A solution of 2.5 g. of 4,5-seco-$\Delta^3$-cholestene-5-one and 1.77 g. of osmium tetroxide in 10 ml. of tetrahydrofuran and 25 ml. of benzene was let stand at room temperature for 2 hr., a solution of 6 g. of sodium sulfite in 80 ml. of water and 150 ml. of ethanol was added, and the mixture was refluxed for 2 hr. on the steam-bath. The hot mixture was filtered and the black residue washed with a little ethanol–benzene and the filtrate concentrated to a small volume and then diluted with water and extracted with ether. The solution, washed with hydrochloric acid and with bicarbonate solution and then dried and evaporated, left a resin of infrared spectrum showing no carbonyl absorption but having a very strong hydroxyl band. This material was dissolved in 5 ml. each of ether and benzene and the solution was treated with 0.2 ml. of boron fluoride etherate and let stand over-

(9) A. Windaus, *Ber.*, **42**, 3774 (1909).

(10) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 65–66.

(11) See ref. 10, pp. 333–334, 344–345.

(12) L. F. Fieser, *Org. Syntheses*, **35**, 36 (1955).

(13) L. F. Fieser, THIS JOURNAL, **75**, 4386 (1953).

(14) Reference 10, pp. 508–509.

(15) This work was done prior to publication of the definitive paper by Clayton, Henbest and Smith[8] and we are indebted to Dr. R. B. Clayton for physical constants and experimental procedures. Our observations are reported because they supplement those later published.

(16) Pl. A. Plattner, H. Heusser and M. Feurer, *Helv. Chim. Acta*, **32**, 587 (1949).

night. When the product was chromatographed on 60 g. of alumina, petroleum ether–benzene (4:1, 3:2) eluates gave 0.9 g. of solid product, m.p. 90–93°. Crystallization from chloroform–methanol to constant m.p. gave material melting at 101–102°, $\alpha_D$ +20° Chf; the infrared spectrum is characterized by the presence of very strong bands at 8.75, 9.55, 9.80 and 11.13 $\mu$.

*Anal.* Calcd. for $C_{27}H_{46}O_2$ (402.64): C, 80.54; H, 11.52. Found: C, 80.81; H, 11.54.

**4,5-Secocholestane-5-one.**—A mixture of 1.03 g. of 4,5-seco-$\Delta^3$-cholestene-5-one and 200 mg. of 10% palladium–charcoal in 50 ml. of ethyl acetate took up one mole equivalent of hydrogen in 0.5 hour. The saturated ketone (negative tetranitromethane test, $\lambda^{Chf}$ 5.85 $\mu$) failed to crystallize but gave a 2,4-dinitrophenylhydrazone which crystallized from chloroform–methanol in orange needles, m.p. 183–184°.

*Anal.* Calcd. for $C_{55}H_{62}O_4N_4$ (568.78): C, 69.68; H, 9.22; N, 9.85. Found: C, 69.59; H, 9.06; N, 9.85.

**4,5-Secocholestane (4).**—Huang-Minlon reduction of the above oily ketone gave an oil, $\alpha_D$ +27° Chf.

*Anal.* Calcd. for $C_{27}H_{50}$ (374.67): C, 86.55; H, 13.45. Found: C, 86.61; H, 13.11.

An apparently identical product, $\alpha_D$ +28°, was obtained from 4,5-seco-$\Delta^3$-cholestene-5-one by Huang-Minlon reduction to an oily hydrocarbon (positive tetranitromethane test, $\lambda^{Chf}$ 6.05, 11.0 $\mu$) and catalytic hydrogenation (palladium–charcoal, ethyl acetate).

**Bile Acid Analog (M. J. Chamberlin).**—Oxidation of 15 g. of methyl $\Delta^5$-cholenate by the procedure previously reported for preparation of ketone 104[1] gave a neutral fraction which on chromatography and crystallization from methanol gave 250 mg. of white spars, m.p. 135–137°, $\alpha_D$ −39.7° Chf, very strong infrared bands at 9.9, 10.1, 10.8 and 11.1 $\mu$; unreactive to Girard reagent at room temperature.

*Anal.* Calcd. for $C_{25}H_{38}O_5$ (418.55): C, 71.74; H, 9.15. Found: C, 71.60; H, 9.07.

### Oxidation Experiments (L. F. F.)

**Ketone 104.**—A 1-l. flask containing 20 g. of Wilson Co. cholesterol and 65 ml. of benzene was heated on the steam-bath until the solution reached the boiling point and then placed on a cork ring. A solution of 150 g. of sodium dichromate (dihydrate) in 470 ml. of acetic acid was adjusted to 70° and poured into the benzene solution and a condenser was put in place. An exothermic reaction set in and benzene refluxed vigorously for 6–7 min. After 10 min. the flask was mounted on a preheated Glas-col heater and the mixture was refluxed for 1 hr. (liquid temperature 100°). The cooled solution was diluted with 250 ml. of water and shaken with a mixture of 130 ml. each of ether and petroleum ether (30–60°). After complete separation of layers (20 min.), the upper layer was washed twice with water containing hydrochloric acid and once with water containing sodium chloride. The solution was filtered into a second funnel through a plug of glass wool to retain a green sludge (acetone soluble) and extracted with three portions of water each containing 10 ml. of 25% sodium hydroxide solution. The total acidic material amounting to 5.0 g. was separated with Girard reagent into a ketonic fraction (1.9 g.) and a non-ketonic fraction (3:1 g.); each was a glassy resin readily soluble in petroleum ether, but neither one afforded crystals. Evaporation of the washed and dried colorless solution of neutral material gave 1.55 g. of an oil which started to solidify while still warm. One crystallization from methanol gave 870 mg. (4.0%) of large needles of ketone 104, m.p. 123–124°. Material recovered from the mother liquor when chromatographed gave early fractions which on crystallization afforded 53 mg. (0.3%) of ketone 104. Later fractions failed to crystallize.

When the reflux time was extended to 2 and to 4 hr. the yield of ketone 104 in the first crop dropped to 845 and to 730 mg. The ketonic acid fraction dropped off slightly (1.5 and 1.4 g.) and the non-ketonic acid fraction fell to 2.6 and to 1.4 g.

The effect of temperature in the initial stages of oxidation was explored in parallel runs like that described except that only 75 g. of dichromate was used initially and the dichromate solution was at 12° and the benzene solution was at room temperature (26°). A paste of cholesteryl chromate which separated initially dissolved in 2 hr., when the temperature had risen from 18 to 42°; it then began to fall. After a total of 20 hr., one solution when worked up afforded 6.2 g. of keto acids and 2.5 g. of non-keto acids; neither fraction could be caused to crystallize. The second solution was treated with 75 g. more sodium dichromate and refluxed for 1 hr. The neutral fraction amounted to 0.77 g. and on crystallization from methanol afforded pure ketone 104 in yield of 273 mg. (1.3%); the total acid fraction weighed 5.0 g.

**Duoannelic Acid (7).**—The mixing of solutions of dichromate (150 g.) and cholesterol (20 g.) was done exactly as in the procedure for preparation of ketone 104. After the initial vigorous reaction had subsided (15 min.), solvent was distilled until the vapor temperature reached 110° (135–140 ml., liquid temperature 121°). The solution was then refluxed for 4 hr. and the mixture worked up as before. The neutral fraction (0.65 g.) gave 240 mg. (1.1%) of crystals of ketone 104. The total acid fraction (3.5 g.) on Girard separation afforded 1.6 g. of non-ketonic acids, but this material failed to crystallize and chromatography after esterification with diazomethane gave no crystalline fractions. The keto acid fraction (1.7 g.) was left on evaporation of the ether as a colorless glass which crystallized on cooling. Digestion with petroleum ether and cooling afforded 0.70 g. of pure duoannelic acid, m.p. 144–145°. Concentration of the mother liquor afforded 180 mg. of satisfactory crystals; yield 3.8%.

Another run was conducted in the same way, but the reflux period was extended to 16 hr. The change was attended with reduction in yield of both neutral fraction (0.50 g.) and total acid fraction (1.4 g.); the keto acid fraction weighed 0.8 g. Duoannelic acid was isolated initially in an experiment similar to the procedure outlined above except that no separation with Girard reagent was made. The total acid fraction (1.7 g.) dissolved readily in 4–5 ml. of petroleum ether and on standing at 5° the solution deposited a mass of crystals consisting largely of duoannelic acid but containing a persistent contaminant removed only on repeated crystallization from petroleum ether; increase in purity is attended with marked decrease in solubility in this solvent. Separation from non-ketonic acids through the Girard derivative thus greatly simplifies isolation of pure duoannelic acid.

**Butenandt Acid.**—A solution of 75 g. of sodium dichromate in 300 ml. of acetic acid was adjusted to 50° and poured into a solution at 50° of 20 g. of cholesterol in 65 ml. of benzene. The precipitated chromate ester rapidly dissolved and the reaction was weakly exothermic for about 30 minutes, during which time the temperature was kept at or slightly below 60° by an occasional brief dip of the flask into water. Once the temperature began to fall, no further attention was required and the solution was let stand overnight. The solution was then diluted and extracted with ether–petroleum ether and the solution was extracted with alkali, exactly as in the preparation of ketone 104. The solution of neutral material (2.5 g.) can be discarded. The dark reddish-brown alkaline solution was brought to *p*H 8 by passing in gaseous carbon dioxide and shaken with a liberal portion of ether, when a dark sludge separated at the interface and could be trapped in the funnel when the considerably clarified bicarbonate layer was drawn off into a clean funnel. Extraction with a second portion of ether removed more sludge, but a third extraction was superfluous. Elimination of this contaminant greatly facilitates crystallization of the final product. The bicarbonate solution was covered with a layer of ether in a beaker and acidified. The acidic material was then collected by ether extraction and obtained as a light yellow resin (8.6 g.). A solution of this material in 50 ml. of ethanol was treated with 4.3 g. of Girard reagent T and 2 ml. of acetic acid and heated for 1 hr. at the boiling point. Dilution with water to a volume of about 130 ml. gave a clear, pale yellow solution which was extracted twice with ether. The aqueous layer was heated on the steam-bath with 5 ml. of concentrated hydrochloric acid, cooled and extracted with ether, and evaporation of the dried extract gave 4.3 g. of resin. This material could not be caused to crystallize by any method other than that previously described,[11] namely, dissolving it in ether and evaporating, with gradual addition of petroleum ether. A main crop of 2.2 g. melted at 179–181°, and a second and third crop brought the yield of satisfactory material to 2.7 g. (12%).

The non-ketonic acid fraction (3.0 g.) from the above separation was a resin which is readily soluble in petroleum ether but which resisted all attempts at crystallization from

this or other solvent. An acetic acid solution of a 3-g. batch from another run was treated with 3 g. of dichromate and refluxed, when it rapidly turned green. Another 3 g. of dichromate was added and the solution was refluxed for a total of 2.5 hr., when it was brownish-green. Dilution and extraction with ether gave 1.0 g. of acid, and this on Girard separation proved to be largely non-ketonic. A small ketonic fraction (0.08 g.), however, crystallized slowly from petroleum ether to give crystals melting at 142–143°, undepressed on admixture with duoannelic acid.

A solution of 5 g. of sodium dichromate in 15 ml. of acetic acid in a test-tube was cooled to 50°, 1.0 g. of pure Butenandt acid was added and washed down with 5 ml. of acetic acid. The tube was then inserted in a flask of refluxing toluene. Vigorous bubbling from evolution of carbon dioxide continued for about 2 hr. and the temperature rose 3.5° above that of the boiler (111°). The oxidation was stopped after 4 hr., when the green solution still showed a yellowish tinge. After extraction with ether the acidic material was extracted from the ether with sodium bicarbonate and obtained as a greenish resin (0.25 g.) which failed to crystallize from petroleum ether. However, Girard separation gave a ketonic fraction (0.14 g.) which crystallized readily from petroleum ether to give 38 mg. of duoannelic acid, m.p. and mixed m.p. 143–144°.

### Characterization (T. G.)

**Duoannelic Acid (7).**—The fully purified acid is a light powder of fine needles, m.p. 145—145.5°, $\alpha_D$ +17.8° Chf.

*Anal.* Calcd. for $C_{21}H_{36}O_3$ (336.50): C, 74.95; H, 10.77. Found: C, 74.90; H, 10.63.

**The methyl ester** (diazomethane) crystallized well from aqueous acetone (twice) in long needles, m.p. 77.5–78.5°. The infrared spectrum showed bands for both an ester and a ketone carbonyl group.

*Anal.* Calcd. for $C_{22}H_{38}O_3$ (350.52): C, 75.38; H, 10.93. Found: C, 75.24; H, 10.93.

**Dihydroduoannelic Acid (10).**—A solution of 500 mg. of duoannelic acid in 30 ml. of methanol was neutralized with 10% potassium hydroxide solution and treated with 150 mg. of sodium borohydride with ice cooling. After standing at room temperature overnight the solution was acidified, diluted, and extracted with ether. The crude product was crystalline, and crystallization from acetone afforded 285 mg. of needles, m.p. 156.5–157.5°. Recrystallized material had the constants m.p. 160–161.5°, $\alpha_D$ +19.5° Chf.

*Anal.* Calcd. for $C_{21}H_{38}O_3$ (338.51): C, 74.51; H, 11.32. Found: C, 74.59; H, 11.43.

**Dihydroduoannelic Acid Lactone (11).**—A solution of 150 mg. of the acid in a mixture of 7.5 ml. of methanol and 2.5 ml. of 10% hydrochloric acid was refluxed for 12 hr., diluted and extracted with ether. Evaporation of the solvent left an oil which soon solidified, and two crystallizations from methanol gave leaflets, m.p. 100.5°, $\alpha_D$ +16.4° Chf, $\lambda^{CS_2}$ 5.62 $\mu$.

*Anal.* Calcd. for $C_{21}H_{36}O_2$ (320.50): C, 78.69; H, 11.32. Found: C, 78.61; H, 11.37.

After shorter periods of refluxing the infrared spectrum indicated that the hydroxy acid had been merely esterified.

**Duoannelic Diacid.**—A suspension of 300 mg. of duoannelic acid in 5 ml. of water was brought into solution by neutralization with 10% potassium hydroxide. A solution prepared by addition of 0.18 ml. of bromine dropwise to an iced solution of 400 mg. of sodium hydroxide in 3 ml. of water and the hypobromite solution was added. After 2 hr. at 0° and 5 hr. at 26° the mixture was treated with 100 mg. of sodium bisulfite and acidified. The solid product that precipitated (m.p. 173–177°) on crystallization from aqueous acetone formed plates, m.p. 179–180.5°.

*Anal.* Calcd. for $C_{20}H_{34}O_2$ (338.47): C, 70.97; H, 10.13. Found: C, 71.22; H, 9.99.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

# Oxido Alcohols and Ketoxides

BY LOUIS F. FIESER AND TOSHIO GOTO[1]

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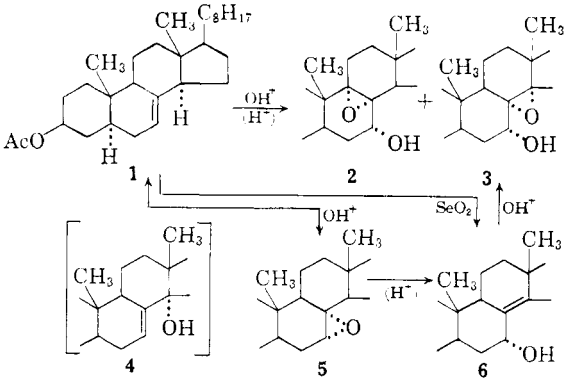
The conversion of $\Delta^7$-cholestenyl acetate (**1**) into the 7$\alpha$-ol-8$\alpha$,14$\alpha$-oxide **3** on reaction with perbenzoic acid in chloroform is shown to involve formation of the 7$\alpha$,8$\alpha$-oxide and its cleavage by a trace of mineral acid to the $\Delta^{8(14)}$-ene-7$\alpha$-ol (**6**). Consideration of the possible mechanism of the formation of ketoxides on chromic acid oxidation of $\Delta^7$- and $\Delta^{8(14)}$-stenyl acetates (*e.g.*, **11** and **13**) suggested revision of the formula (**21a**) assigned by Ellis and Petrow to an alcohol resulting from selenium dioxide oxidation of Westphalen's diol. Evidence from ultraviolet and nuclear magnetic resonance spectra support the new formula **21b**.

An observation of Wintersteiner and Moore[2] that has not yet been clarified is that $\Delta^7$-cholestenyl acetate (**1**) reacts with two moles of perbenzoic acid in chloroform to give in good yield a mixture of the 8$\alpha$,9$\alpha$- and 8$\alpha$,14$\alpha$-oxido-7$\alpha$-ols **2** (minor product) and **3**,[3] of stereochemistry later deduced in our laboratory.[4] Fieser and Ourisson[4] found that the $\Delta^7$-compound **1** reacts with selenium dioxide in acetic acid or ethanol to give the $\Delta^{8(14)}$-ene-7$\alpha$-ol (**6**), isolated either as the acetate or the ethyl ether, and suggested that the oxido alcohol **3** may arise in the reaction with perbenzoic acid by allylic hydroxylation at $C_{14}$ (**4**), allylic rearrangement to **6**, and oxide formation. Indeed Saucy, *et al.*,[5] isolated the analogous $\Delta^7$-ene-9-ol along with




the $\Delta^{8(14)}$-ene-7$\alpha$-ol on oxidation of 5-dihydroergosteryl acetate with selenium dioxide. However, there is no evidence that a peracid can effect allylic hydroxylation, and indeed two $\Delta^7$-stenols

(1) Recipient of a Fulbright travel grant on leave from Nagoya University, Nagoya, Japan.

(2) O. Wintersteiner and M. Moore, THIS JOURNAL, **65**, 1507 (1943).

(3) Characterization of **3**: L. F. Fieser, K. Nakanishi and W.-Y. Huang, *ibid.*, **75**, 4719 (1953).

(4) L. F. Fieser and G. Ourisson, *ibid.*, **75**, 4404 (1953).

(5) G. Saucy, P. Geistlich, R. Hebling and H. Heusser, *Helv. Chim. Acta*, **37**, 250 (1954).