[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN COMPANY, KALAMAZOO, MICHIGAN]

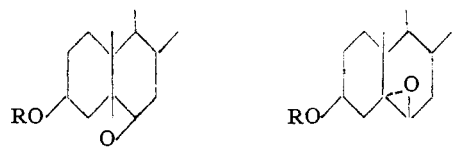
Studies on Cholesteryl Oxides[1]

BY PURNENDU N. CHAKRAVORTY AND ROBERT H. LEVIN

In connection with work in progress, it became necessary to study the properties of the oxide ring in cholesteryl-5,6-oxides. Previous investigators have used perbenzoic acid to prepare the various oxido derivatives of cholesterol, and from their results the nature of the substituent at C_3 appeared to influence the ratio of stereoisomeric products formed. Thus from cholesterol Westphalen and Windaus[2] obtained α-cholesterol oxide (I, R = H) in 50% yield, with some of the β-isomer (II, R = H) being isolable from the crystallization mother liquors. Ruzicka and Bosshard[3] found that treatment of cholesteryl acetate with perbenzoic acid produced largely β-cholesteryl oxide acetate. Spring and Swain[4] reported that cholesteryl benzoate, under the same conditions, formed almost equal amounts of α- and β-cholesteryl oxide benzoates in excellent yield.

In this Laboratory, preparation of oxides has been simplified by the use of monoperphthalic acid.[5] This reagent is readily prepared and is more stable than perbenzoic acid. Further, the phthalic acid which is formed from the perphthalic acid during the reaction can be separated easily from the sterol oxides because of its insolubility in chloroform. Such ease of separation is particularly useful in the preparation of bile acid oxides because it eliminates the need for washing with alkali. However, when using perphthalic acid several precautions are necessary. The chloroform should be freshly distilled over potassium carbonate, since the sterol oxides will react with even traces of hydrogen chloride. In some of our experiments, the perphthalic acid–cholesteryl compound reaction mixture was not worked up immediately but allowed to stand overnight or longer. In these instances the reaction product was quite sticky and uncrystallizable, and the yield was small. This was presumably due to a secondary reaction of the cholesteryl oxide with phthalic acid, giving, very probably, a half phthalic ester as was indicated by the solubility of the product in alkali.

When cholesteryl acetate was treated with perphthalic acid, β-cholesteryl oxide acetate (II, R = Ac) was isolated in 50% yield and the α

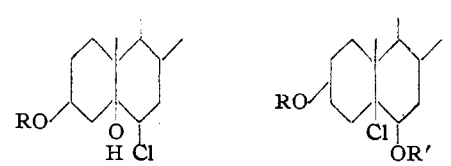


I[6] II[6]

R = H, Ac, Bz

isomer (I, R = Ac) in 20% yield. A similar reaction using cholesteryl benzoate gave α-cholesteryl oxide benzoate (I, R = Bz) in 50 to 70% yield, but no β-oxide benzoate could be obtained. With cholesterol, the same reagent produced α-cholesterol oxide (I, R = H) in 60% yield, and a small amount of the β-isomer.

Several partially successful attempts to rearrange cholesteryl oxides to ketocholestane compounds have been reported. Chinaeva and Ushakov[7] used a Grignard reagent. Spring and Swain[4] employed heat and vigorous dehydrating reagents. In neither instance was the yield of 6-ketocholestanol satisfactory. It was thought that any tendency of the oxido group to isomerize into a ketone might be accelerated through a mass action effect by removing the product from the sphere of reaction as a ketone derivative. Accordingly, β-cholesteryl oxide acetate was heated with semicarbazide hydrochloride in pyridine. The product of this reaction was a halogen containing substance, m. p. 188–190°, which could be recovered unchanged after refluxing with acetic anhydride, and was therefore identified as a 3-acetoxy-5-hydroxy-6-chlorocholestane (III, R = Ac) instead of 3-acetoxy-5-chloro-6-hydroxycholestane (IV, R = Ac, R′ = H). A rational explanation of this reaction became possible when it was found



III IV

(1) Presented in part before the Organic Division of the American Chemical Society, Memphis, April 20–24, 1942.

(2) Westphalen and Windaus, *Ber.*, **48**, 1064 (1915).

(3) Ruzicka and Bosshard, *Helv. Chim. Acta*, **20**, 244 (1937).

(4) Spring and Swain, *J. Chem. Soc.*, 1356 (1939).

(5) For preparation of the reagent see Böhme, *Ber.*, **70**, 379 (1937); "Organic Syntheses," **20**, 70 (1940).

(6) Throughout this paper the steric configurations assigned at C_3 and C_5 are purely arbitrary.

(7) Chinaeva and Ushakov, *J. Gen. Chem.* (U. S. S. R.), **11**, 335 (1941); *C. A.*, **35**, 5903.

that a solution of semicarbazide hydrochloride in pyridine has a *p*H of 6.2 and will turn blue litmus red. Since ethylene oxides are, in general, stable to alkaline reagents, but susceptible to acidic substances, this transformation is not surprising.

It has been known for some time that ethylene oxide rings in aliphatic molecules are also opened by heavy metal and even alkaline earth metal halides.[8] In an extension of this reaction to cholesteryl oxides, ferric chloride, zinc chloride and magnesium bromide[9] were found to react with β-cholesteryl oxide acetate to give compounds containing halogen. The ferric chloride reaction product was identified as 3-acetoxy-5-hydroxy-6-chlorocholestane (III, R = Ac). The zinc chloride and magnesium bromide products were not completely characterized.

As indicated above, cleavage of the cholesteryl oxide bond can produce two position isomers (III, IV). Recently some conflicting reports have been published concerning the orientation of products obtained by the action of certain acidic reagents on various cholesteryl oxide derivatives. Ruzicka and Bosshard[3] treated the β-oxide acetate with dry hydrogen chloride in pyridine and obtained 3-acetoxy-5-hydroxy-6-chlorocholestane (III, R = Ac). Hattori[10] reported this same compound resulting from the action of hydrogen chloride on α-cholesteryl oxide acetate, and claimed that very pure β-oxide acetate[11] gave a 5-chloro-6-hydroxycholestane derivative (IV, R = Ac, R′ = H). Spring and Swain[4] have found that α-cholesterol oxide and its benzoate react with benzoyl chloride in pyridine or with hydrogen chloride to give 5-hydroxy-6-chlorocholestane compounds (III), whereas β-cholesterol oxide and its benzoate form 5-chloro-6-hydroxy derivatives (IV, R = Bz, R′ = Bz, H). We treated β-cholesteryl oxide acetate, m. p. 113°, with benzoyl chloride in pyridine and obtained 3-acetoxy-5-hydroxy-6-chlorocholestane (III, R = Ac), which was characterized by mixed melting point, analysis, and its non-reactivity toward acetic anhydride. If the action of benzoyl chloride involves simply a cleavage of the cholesteryl oxide linkage followed by addition of fragments of the benzoyl chloride molecule, then in our reaction, as well as Spring and Swain's reaction with the α-oxide and its benzoate, the 5-benzoxy compound would be expected to form.[12] Since the 5-hydroxy derivative was obtained, it seems that the benzoyl chloride acts like the semicarbazide hydrochloride, that is, merely as a donor of hydrogen chloride. Formation of 6-benzoxy compounds can be explained on the assumption that the primary reaction is fission by hydrogen chloride, followed by benzoylation of the newly formed 6-hydroxy group with excess of the reagent.

It was thought that pyridine might function as a carrier of hydrogen chloride in these experiments. Therefore, the reaction of "pyridine hydrochloride" with β-cholesteryl oxide acetate was undertaken. This reagent was obtained as a hygroscopic white solid by passing dry hydrogen chloride into a solution of pyridine in ether. In petroleum ether there was no reaction, "pyridine hydrochloride" being insoluble in this solvent. In absolute ethanol a reaction readily took place, with the formation of the same 3-acetoxychlorohydrin (III) previously obtained with semicarbazide hydrochloride, ferric chloride and benzoyl chloride.

The reaction of β-cholesteryl oxide acetate with benzoyl chloride was also carried out under anhydrous conditions using carbon tetrachloride as a solvent. The substitution of a completely chlorinated solvent for pyridine, which contains hydrogen, did not alter the course of the reaction, and the same 5-hydroxy-6-chlorocholestane derivative was isolated from the reaction mixture although in a poorer yield. Apparently the elements of hydrogen chloride are obtained from the benzoyl chloride, but the mechanism of the transformation remains quite obscure.

The reaction with "pyridine hydrochloride" was extended to α-cholesterol oxide, its acetate, and benzoate; and β-cholesterol oxide. These substances, to our surprise, uniformly gave 5-hydroxy-6-chlorocholestane derivatives (III) in good yields. "Pyridine hydrochloride" is a convenient reagent to use. It is easily prepared, may be kept indefinitely in a desiccator, reacts smoothly, and any excess is readily disposed of. In the case of α- and β-cholesterol oxides, some trouble was experienced in purifying the reaction products be-

(8) Bodforss, "Die Äthylenoxyde," *Sammlung chem. u. chem. techn. Vortrage*, Ferdinand Enke, Stuttgart, 1920.

(9) Ushakov and Madaeva, *J. Gen. Chem.* (U. S. S. R.), **9**, 1690 (1939), treated α-cholesterol oxide with magnesium bromide, but did not isolate any compound containing halogen.

(10) Hattori, *J. Pharm. Soc. Japan*, **60**, 334 (1940).

(11) Hattori's value for the m. p. of β-cholesterol oxide, 136°, is at variance with all other published values and our own findings which give a m. p. of 106–107° for the pure substance. However, his oxide acetate has the accepted m. p., 112–113°.

(12) See for example Ehrenstein's acetolysis of the stereoisomeric 5,6-oxides of dehydroandrosterone acetate, *J. Org. Chem.*, **6**, 626 (1941); also Hattori, ref. 10.

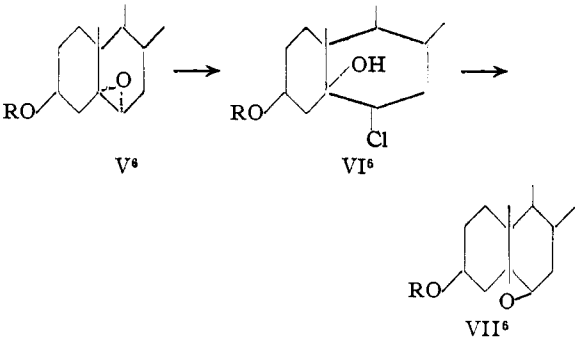
cause of dehalogenation occurring during recrystallization. The difficulty was obviated by benzoylating the crude dry reaction product and then crystallizing. Apparently the presence of a free hydroxyl group at C_3 labilizes the halogen at C_6, forming possibly a 3,6-oxide.

From the results of previous investigators it appeared that the configuration of the oxide and the nature of the substituent at C_3 affected the orientation of the chlorohydrin produced by fission of the oxide ring. Our experiments, however, indicated that the reagent might become a dominant factor in determining the course of the reaction. It became desirable to check Spring and Swain's experiments with benzoyl chloride in pyridine. Using α-cholesterol oxide, its acetate and benzoate, and β-cholesterol oxide, the products again were derivatives of 5-hydroxy-6-chlorocholestane (III). In the reaction of β-cholesterol oxide, a new substance was obtained together with 3-benzoxy-5-hydroxy-6-chlorocholestane. The new product has a m. p. of 197–198° and gave a sharp depression with the 3-benzoxy chlorohydrin obtained above. It was not, however, the expected dibenzoate of 3,6-dihydroxy-5-chlorocholestane, since that compound has a melting point of 184°. Work is in progress to elucidate its structure.

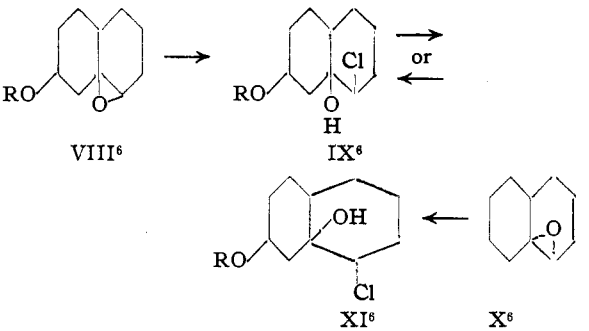
Thus Spring and Swain obtained 5-hydroxy-6-chloro compounds from the α-oxide series and 5-chloro-6-hydroxy derivatives from the β-oxides, while we could isolate only one type, regardless of which oxide we used. Since the experimental procedures were basically similar, this discrepancy, at the moment, seems difficult to explain.

Mild saponification of 3-acetoxy-5-hydroxy-6-chlorocholestane (III, R = Ac) with sodium carbonate gave α-cholesterol oxide, m. p. 141°. This is in accordance with the work of Spring and Swain,[13] who obtained α-cholesteryl oxide benzoate by heating 3-benzoxy-5-hydroxy-6-chlorocholestane (III, R = Bz) with quinoline. Previously Windaus[14] had reported that the 5-chloro compound, 3,6-dihydroxy-5-chlorocholestane, forms β-cholesterol oxide when treated with alkali.

The stereochemical aspects of these reactions should be pointed out. In the above case, for example, an oxide with a β-configuration (V) combined with the elements of hydrogen chloride to form a 5-hydroxy-6-chloro compound (VI),



which, on losing hydrogen chloride, gave an oxide of the opposite configuration (VII). Windaus[14] started with an α-oxide and obtained a 5-chloro-6-hydroxycholestane derivative which lost halogen acid and formed a β-oxide. Furthermore, we have obtained the same chlorohydrin from oxides of different configuration. The current theory states that in the cleavage of an oxide linkage a *trans*-compound is formed.[15] However, if an α-oxide (VIII) forms a *trans*-5-hydroxychlorohydrin (IX), and a β-oxide (X) similarly produces a *trans*-5-hydroxy compound (XI), then the two will neces-



sarily be of opposite configuration at C_5,C_6. Even assuming that the α-oxide may give a 5-hydroxy-6-chloro derivative of a geometrical configuration different from IX, then the β-oxide, following the same route, would still give the opposite stereoisomer. Thus, it is necessary to postulate additional Walden inversions in one case but not in the other or an entirely different mechanism to explain how the same steric configuration is obtained by cleavage of the stereoisomeric oxides of cholesterol, or how the stereoisomers are interconverted through an intermediate chlorohydrin.

Further studies on the oxides of cholesterol will be reported in a subsequent communication.

(13) Spring and Swain, *J. Chem. Soc.*, 83 (1941).

(14) Windaus, *Z. physiol. Chem.*, **117**, 154 (1921).

(15) Lucas, Schlatter and Jones, THIS JOURNAL, **63**, 22 (1941), describe the opening of the oxide rings in the isomeric 2,3-epoxypentanes. See also Ehrenstein, *J. Org. Chem.*, **4**, 506 (1939); and Ehrenstein and Decker, *ibid.*, **5**, 544 (1940).

Experimental[16]

Monoperphthalic acid was prepared according to the method of Böhme[5] and kept in the refrigerator for as long as a month without appreciable decomposition.

β- and α-Cholesteryl Oxide Acetates.—Ten grams (0.023 mole) of cholesteryl acetate, m. p. 112–114°, dissolved in 50 cc. of ether, was mixed with 8.4 g. (0.046 mole) of monoperphthalic acid in 266 cc. of ether. The solution was allowed to reflux for six hours and the solvent removed by distillation. The residue was dried *in vacuo* and digested with 250 cc. of chloroform dried over potassium carbonate. Filtration gave a residue of 6.7 g. (87% recovery) of phthalic acid and a clear colorless solution which was taken to dryness *in vacuo*. The residue was crystallized from 30 cc. of methanol, giving 6.0 g. (58%) of β-cholesteryl oxide acetate, which on recrystallization from methanol gave the pure product, m. p. 111–112°, $[\alpha]^{25}D$ −21.8°; mixed m. p. with cholesteryl acetate, 97–101°. Concentration of the filtrate gave 1.55 g. (15%) of α-cholesteryl oxide acetate. The α-isomer was purified by crystallization from ethanol and found to have a m. p. of 101–103°, $[\alpha]^{25}D$ −44.6°. The m. p. is 5° higher than previously reported values.[2,10] However, it gave a depression (m. p. 88–104°) when mixed with pure β-oxide acetate. Saponification with methanolic potassium hydroxide and crystallization from dilute alcohol gave α-cholesterol oxide, m. p. 141–143°, $[\alpha]^{25}D$ −44.5°.[2]

In several runs the reaction mixture was left over the week-end. The yield of oxides in these instances was very poor and a considerable amount of alkali soluble substance was formed. Since this product remained dissolved in alkali after saponification, it is assumed to be a 5-half-phthalate derivative of cholestane.

α-Cholesteryl oxide benzoate was similarly prepared. Cholesteryl benzoate (16.7 g., 0.034 mole) reacting with perphthalic acid (12.7 g., 0.07 mole) gave 8.7 g. (50%) of α-cholesteryl oxide benzoate, m. p. 164–166°, $[\alpha]^{25}D$ −28.0.[4] No β-oxide benzoate could be isolated.

α-Cholesterol Oxide.—In the same manner, 5 g. (0.013 mole) of cholesterol (Wilson) and 4.7 g. (0.026 mole) of perphthalic acid gave 3.1 g. (61%) of α-cholesterol oxide, m. p. 141–143°. A small amount of the β-isomer was obtained from the mother liquors.

β-Cholesterol oxide, m. p. 105–107°, $(\alpha)^{25}D$ −12.7, was also obtained by saponification of the β-oxide acetate.

Reaction of β-Cholesteryl Oxide Acetate with Semicarbazide Hydrochloride.—A solution of 2.3 g. of β-cholesteryl oxide acetate in 10 cc. of pyridine was treated with 2.0 g. of semicarbazide hydrochloride and warmed on the steam-bath for six hours. The solution was concentrated to half its volume and poured over crushed ice. The precipitate was separated by filtration, digested with methanol, and filtered while hot to remove a small amount of disemicarbazide. Concentration and cooling gave colorless needle-like crystals, m. p. 183–186°, which gave a strong Beilstein test. These were combined with crystals recovered from the mother liquor and recrystallized twice from dilute acetone, giving 0.8 g. of cholesteryl acetate chlorohydrin, m. p. 187.5–189.5°.

Anal. Calcd. for $C_{29}H_{49}O_3Cl$: C, 72.4; H, 10.3; Cl, 7.38. Found: C, 72.2; H, 10.1; Cl, 7.31.

(16) Microanalyses by H. Emerson and W. Struck.

A portion (200 mg.) of the chlorohydrin, refluxed for fifteen minutes with acetic anhydride, was recovered unchanged, hence it was characterized as 3-acetoxy-5-hydroxy-6-chlorocholestane (III).

β-Oxide Acetate and Ferric Chloride.—β-Oxide acetate (0.40 g.) was treated with 1.0 g. of ferric chloride in alcohol. The solution was allowed to reflux for three hours, then concentrated to half its volume and water added. Ether extraction and isolation in the usual manner gave a chlorohydrin which was purified by repeated crystallization from dilute methanol and dilute acetone. The final product melted at 183–185°, and was recovered unchanged after refluxing with acetic anhydride. There was no depression of the m. p. on admixture with authentic 3-acetoxy-5-hydroxy-6-chlorocholestane.

β-Oxide Acetate and Benzoyl Chloride. 1. **In Pyridine.**—To a solution of 0.45 g. of β-cholesteryl oxide acetate in 5 cc. of dry pyridine was added 0.5 cc. of benzoyl chloride. The mixture was allowed to stand at room temperature for one-half hour, then heated on the steam-bath for the same period. The solution was poured into ice water and the semi-crystalline precipitate separated by decantation. Crystallization from dilute methanol gave 0.42 g. of chlorohydrin, m. p. 150–161°. Two recrystallizations from dilute acetone gave 0.25 g. of 3-acetoxy-5-hydroxy-6-chlorocholestane, m. p. 183–185°, which showed no depression of the m. p. on admixture with the product of the semicarbazide hydrochloride reaction.

Anal. Calcd. for $C_{29}H_{49}O_3Cl$: C, 72.4; H, 10.3; Cl, 7.38. Found: C, 72.3; H, 10.7, Cl, 7.26.

The compound was recovered unchanged after refluxing with acetic anhydride.

2. **In Carbon Tetrachloride.**—A solution of 0.60 g. of β-oxide acetate in 30 cc. of carbon tetrachloride distilled over potassium carbonate was treated with 0.50 cc. of benzoyl chloride under anhydrous conditions. The solution was allowed to reflux for three hours, then kept at room temperature for thirty-six hours. After distilling the solvent, the excess benzoyl chloride was removed *in vacuo*. Crystallization from dilute methanol gave a product which sintered at 100° and melted at 135–155°. A Beilstein test for halogen was positive. Four crystallizations from dilute acetone gave colorless needles of 3-acetoxy-5-hydroxy-6-chlorocholestane, m. p. 182–185°. There was no depression of the m. p. on admixture with an authentic sample.

Anal. Calcd. for $C_{29}H_{49}O_3Cl$: C, 72.4; H, 10.3; Cl, 7.38. Found: C, 72.3; H, 10.2; Cl, 7.17.

β-Oxide Acetate and "Pyridine Hydrochloride."—**"Pyridine Hydrochloride"** was prepared by passing dry hydrogen chloride into a solution of pyridine in ether and allowing the saturated solution to stand for one-half hour. The white solid was separated by filtration and found to be very hygroscopic. It is insoluble in dioxane and petroleum ether and soluble in alcohol. Crystallization from absolute alcohol–ether gave colorless platelets, m. p.: s. 81; m. 141–146°. It can be kept for months in a desiccator over calcium chloride with no evidence of decomposition.

To a solution of 1.0 g. of β-cholesteryl oxide acetate in 30 cc. of absolute ethanol was added 1.0 g. of pyridine hydrochloride. The solution was allowed to reflux for fifteen

minutes, then concentrated to half its volume and the sterol precipitated by the addition of water. Crystallization from dilute acetone gave 0.85 g. (77%) of crude 3-acetoxy-5-hydroxy-6-chlorocholestane, m. p. 148–168°. A Beilstein test was positive. After several crystallizations from dilute acetone, colorless needles, m. p. 184–186° were obtained. The product was recovered unchanged after treatment with acetic anhydride, and gave no depression in m. p. on admixture with authentic 3-acetoxy-5-hydroxy-6-chlorocholestane.

Reaction of Pyridine Hydrochloride with the Other Oxides of Cholesterol.—α-Cholesteryl oxide benzoate is sparingly soluble in alcohol, so it was necessary to dissolve it in benzene. One gram of the oxide in benzene was mixed with 2.0 g. of pyridine hydrochloride in ethanol. The solution was refluxed for fifteen minutes, the solvent evaporated, and the reaction product crystallized from methanol as a halogen containing compound, m. p. 193–196°. Recrystallization from ethyl acetate–methanol, and dilute acetone gave 0.60 g. (56%) of 3-benzoxy-5-hydroxy-6-chlorocholestane, m. p. 196–198°. It was recovered unchanged after refluxing with acetic anhydride.

Anal. Calcd. for $C_{34}H_{51}O_3Cl$: C, 75.2; H, 9.46; Cl, 6.53. Found: C, 75.0; H, 9.40; Cl, 6.33.

α-Cholesteryl oxide acetate (0.80 g.) and 1.0 g. of pyridine hydrochloride in alcohol gave 0.65 g. (74%) of 3-acetoxy-5-hydroxy-6-chlorocholestane, m. p., and mixed m. p., 185–187°. It was recovered unchanged after treatment with acetic anhydride.

α- and β-Cholesterol oxides also reacted smoothly with pyridine hydrochloride. However, the product lost halogen easily, thereby hindering its purification. Thus 0.30 g. of the **α-oxide** gave 0.25 g. of crude chlorohydrin, m. p. 154–160°; Beilstein test positive. Recrystallization from methanol–acetone gave feathery needles, m. p. 99–105°. This product no longer contained halogen.

Benzoylation of the crude product gave an easily purifiable substance. Using 0.45 g. of α-oxide and 1.0 g. of pyridine hydrochloride, a chlorohydrin was obtained. It was dried and benzoylated with 0.50 cc. of benzoyl chloride in pyridine. The product was isolated in the usual way and crystallized from ethyl acetate–methanol, giving 0.35 g. (58%) of 3-benzoxy-5-hydroxy-6-chlorocholestane, m. p. 193–196°. Recrystallization from dilute acetone, and again from ethyl acetate–methanol, gave colorless needles, m. p. 196–198°, which showed no depression on admixture with an authentic sample.

Similarly, from 0.40 g. of the **β-oxide** there was obtained 0.28 g. (52%) of 3-benzoxy-5-hydroxy-6-chlorocholestane, m. p. 192–195°. When mixed with an authentic sample the m. p. was not depressed.

Reaction of Benzoyl Chloride with the Other Oxides of Cholesterol.—Reactions with benzoyl chloride did not proceed as smoothly as the corresponding reactions with pyridine hydrochloride and the products were less pure. Treatment of 0.70 g. of **α-oxide** acetate with 0.60 cc. of benzoyl chloride gave 0.42 g. (55%) of a product, m. p. 160–170°. After heating with acetic anhydride and recrystallizing twice from dilute acetone, 3-acetoxy-5-hydroxy-6-chlorocholestane was obtained as colorless needles, m. p. 178–183°. A mixed m. p. with authentic acetoxychlorohydrin, however, was 179–184°.

Anal. Calcd. for $C_{29}H_{49}O_3Cl$: C, 72.4; H, 10.3. Found: C, 72.6; H, 10.1.

Starting with 0.50 g. of **α-oxide benzoate**[4] and 0.50 cc. of benzoyl chloride, 0.45 g. (84%) of 3-benzoxy-5-hydroxy-6-chlorocholestane, m. p. 191–195°, was obtained. Recrystallization from dilute acetone gave a pure product, m. p. 197–198.5°. A mixed m. p. with authentic benzoate chlorohydrin gave no depression.

Anal. Calcd. for $C_{34}H_{51}O_3Cl$: C, 75.2; H, 9.46; Cl, 6.53. Found: C, 75.0; H, 9.29; Cl, 6.46.

Using 0.15 g. of **α-cholesterol oxide** and 0.20 cc. of benzoyl chloride, the benzoate chlorohydrin was obtained in fair yield. After several recrystallizations its m. p. and mixed m. p. with authentic 3-benzoxy-5-hydroxy-6-chlorocholestane was 193–196°.

The reaction of **β-cholesterol oxide** with benzoyl chloride was also carried out according to Spring and Swain.[4] The results were, however, different, so the details of the experiment are given. The **β-oxide** (0.30 g.) in 4 cc. of dry pyridine was warmed for an hour with 0.50 cc. of benzoyl chloride. The solution was poured into ice-water and the precipitate separated by filtration. The residue was crystallized from 30 cc. of acetone–methanol, giving 0.15 g. of 3-benzoxy-5-hydroxy-6-chlorocholestane, m. p. 190–193°. Recrystallizations from acetone–methanol and dilute acetone brought the m. p. up to 195–197°.

Anal. Calcd. for $C_{34}H_{51}O_3Cl$: C, 75.2; H, 9.46; Cl, 6.53. Found: C, 74.9; H, 9.50; Cl, 6.47.

Concentration of the first mother liquor gave 0.14 g. of a crystalline compound, m. p. 176–182°. It was recrystallized twice from acetone–methanol, in which it seemed to be more soluble than the previously obtained benzoate chlorohydrin. It had a m. p. of 197–198°, and its mixed m. p. with authentic 3-benzoxy-5-hydroxy-6-chlorocholestane was 179–186°. This compound is not 3,6-dibenzoxy-5-chlorocholestane.

Anal. Calcd. for 3,6-dibenzoxy-5-chlorocholestane, m. p. 184°, $C_{41}H_{55}O_4Cl$: Cl, 5.48. Found: Cl, 7.01, 7.11.

α-Oxide from 5-Hydroxy-6-chlorocholestane Derivatives.—A solution of 0.7 g. of 3-acetoxy-5-hydroxy-6-chlorocholestane in 30 cc. of dilute alcohol was treated with 10 cc. of 2 *N* sodium carbonate and warmed on the steam-bath for thirty minutes. The addition of water gave a precipitate of 0.40 g. of **α-cholesteryl oxide** which was recrystallized twice from ethyl acetate, m. p. 141–143°. A mixed m. p. with an authentic sample of α-oxide showed no depression.

A similar reaction was carried out with 3-benzoxy-5-hydroxy-6-chlorocholestane and methanolic potassium hydroxide, again giving α-cholesterol oxide, m. p. 141–143°.

Summary

α- and β-cholesterol oxide, α- and β-cholesteryl oxide acetate and α-cholesteryl oxide benzoate have been conveniently prepared through the action of perphthalic acid on cholesterol and its derivatives.

Semicarbazide hydrochloride and ferric chloride react with β-cholesteryl oxide acetate to form 3-acetoxy-5-hydroxy-6-chlorocholestane.

All five of the cholesteryl oxides prepared react with "pyridine hydrochloride" and benzoyl chloride to give 5-hydroxy-6-chlorocholestane derivatives.

β-Cholesterol oxide, as its acetate, has been converted to α-cholesterol oxide through the 5-hydroxy-6-chloro compound.

The stereochemical implications of these reactions have been pointed out.

KALAMAZOO, MICHIGAN RECEIVED JULY 17, 1942

[CONTRIBUTION FROM THE MEDICAL-RESEARCH DIVISION, SHARP AND DOHME, INC.]

Synthesis of *p*-Hydroxyphenyl Amyl Sulfide

BY ELLIS MILLER, FRANK S. CROSSLEY AND MAURICE L. MOORE

A previous publication from these Laboratories[1] confirmed the report[2] that the hydroxyphenyl alkyl sulfides were more powerful in their bactericidal activity than the corresponding alkylphenols. These results prompted us to seek a practical method for the preparation of *p*-hydroxyphenyl *n*-amyl sulfide, which had been shown to possess maximum activity for the compounds in this series.

The hydroxydiphenyl sulfides have been prepared by Hilbert and Johnson[3] by use of the Ziegler[4] reaction between diazotized anisidine and thiophenol. The diazothio ether thus formed breaks down at 70° to give a methoxydiphenyl sulfide which is converted into the desired product by dealkylation. They reported that an attempt to prepare *p*-hydroxydiphenyl sulfide by treating diazotized *p*-aminophenol with sodium thiophenate was unsuccessful. Suter and Hansen[2] treated diazotized anisidine with potassium ethyl xanthate and decomposed the intermediate diazonium ethyl xanthate to obtain *p*-methoxythiophenol which then reacted with amyl bromide and the product was dealkylated to give *p*-hydroxyphenyl *n*-amyl sulfide. They also reported an unsuccessful attempt to combine the sodium salt of butanethiol-1 with diazotized *p*-aminophenol.

In the previous publication,[1] a more satisfactory procedure was developed which involved the synthesis of thiohydroquinone and subsequent reaction of this with amyl bromide to give the desired *p*-hydroxyphenyl *n*-amyl sulfide. The success of the thiohydroquinone synthesis suggested that diazotized *p*-aminophenol should react with the sodium salt of *n*-amyl mercaptan, even though previous workers[2,3] had been unsuccessful with similar reactions. An extensive study of possible experimental conditions for the reaction has shown that it can be used to prepare the compound in yields of 25–30%.

The diazotization was carried out at a temperature of 0–10° and the diazothio ether decomposed by heating at 60°. The resulting product was distilled and gave a yield of 50–60% of a material which solidified at room temperature. However, on crystallizing the material from solvent naphtha the yield of *p*-hydroxyphenyl *n*-amyl sulfide dropped to 25–30%. Careful fractionation of the reaction mixture disclosed that two definite products were formed during the reaction and permitted the isolation of the second product with its purification. The product was identified by preparation of derivatives and synthesis as di-*n*-amyl disulfide. The reaction has been applied to *n*- and isoamyl mercaptan with comparable results.

Experimental Part

***p*-Hydroxyphenyl *n*-Amyl Sulfide.**—*p*-Aminophenol, 21.8 g. (0.2 mole), was dissolved in 110 cc. of 4 *N* hydrochloric acid (0.44 mole) and diazotized in the usual manner by the slow addition of a solution of 15 g. (0.23 mole) of sodium nitrite in 30 cc. of water. The mixture was stirred until the diazotization was complete (starch–iodide paper test) and the deep purple solution of *p*-hydroxyphenyldiazonium chloride rapidly filtered from a small amount of insoluble material. The solution was then added slowly to a cold (10°) sludge of 24 g. (0.23 mole) of *n*-amyl mercaptan in 75 cc. of water containing 37.5 g. (0.94 mole) of sodium hydroxide. The temperature was maintained at 10° throughout the addition and frothing was controlled by the addition of small amounts of *n*-butanol. After the reaction had subsided, the cooling bath was removed and stirring continued at room temperature until the diazonium salt disappeared (R-salt test). The mixture was then heated to 60° to complete the decomposition of the diazothio ether and allowed to stand overnight at room temperature, after which it was diluted with five volumes of water, acidified with concentrated hydrochloric acid and extracted with 500 cc. of toluene. The extract was washed three times with 500-cc. portions of water, dried over anhydrous

(1) Miller and Read, THIS JOURNAL, **55**, 1224 (1933).
(2) Suter and Hansen, *ibid.*, **54**, 4100 (1932).
(3) Hilbert and Johnson, *ibid.*, **51**, 1526 (1929).
(4) Ziegler, *Ber.*, **23**, 2469 (1890).