to 21.7 g. of free desoxycholic acid per 100 g. of sheep bile concentrate. By the usual methods of separation, 100 g. of concentrate yields about 4.4 g. of desoxycholic acid and 16.8 g. of cholic acid, convertible into 10.9 g. of desoxycholic acid by our process to give a total of 15.3 g. of the acid.

Summary

An improved method for the conversion of cholic acid into desoxycholic acid has been found in oxidation of the free acid at C_7 with N-bromosuccinimide in aqueous bicarbonate solution, followed by Wolff–Kishner reduction according to Huang-Minlon; the over-all yield is 68%. The oxidizing agent is more selective than chromic acid, bromine or even N-bromoacetamide, for the alcoholic groups at C_3 and C_{12} remain unattacked in the presence of an excess. In consequence, desoxycholic acid can be prepared with greater efficiency and ease than heretofore by direct application of the procedure to the total crude acids of saponified bile.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Selective Oxidation with N-Bromosuccinimide. II. Cholestane-3β,5α,6β-triol[1]

BY LOUIS F. FIESER AND SRINIVASA RAJAGOPALAN[1]

On exploring further possible applications of a method of oxidation found particularly effective for the selective oxidation of the 7α-hydroxyl group of cholic acid, we found that cholesterol (I) on oxidation with N-bromosuccinimide in aqueous acetone is converted in moderate yield into cholestane-3β,5α-diol-6-one (III).[2] The reaction could conceivably proceed through an intermediate oxide, but cholesterol α-oxide (Va) under the same conditions was found to yield a mixture containing only a small amount of the diolone III together with cholesterol 5,6-dibromide, and a bromo α,β-unsaturated ketone of analysis and absorption spectrum consistent with formula VI. We then found that cholestane-3β,5α,6β-triol can be oxidized to the diolone III in extraordinarily high yield in aqueous dioxane, acetone, or even methanol–ether or methanol. The triol II is known to be attacked preferentially at C_6 on chromic acid oxidation,[3] but an oxidation conducted by adding the reagent gradually over a ten-hour period afforded the 6-ketone as 3-acetate in only 65% yield, for without careful control the 3,6-diketone is easily formed.[2] No control is required in the present procedure, for the same high yield was obtained with 2.1 as with 1.05 equivalents of N-bromosuccinimide.

The best previous methods for preparation of cholestane-3β,5α,6β-triol are by reaction of cholesterol on the 3-acetate with hydrogen peroxide in acetic acid over a period of four days and saponification of the resulting acetate mixture, or hydrolysis of cholesterol α,β-oxide mixture.[2,4,5] Cleavage of the oxides by acetolysis with organic acids and hydrolysis with dilute sulfuric acid are attended with extensive or partial ester formation. An interesting incidental observation is that both cholesterol α-oxide and cholesteryl α,β-oxide acetate can be cleaved in high yield to the triol II or its 3-acetate by the action of periodic acid in refluxing aqueous acetone. This acid apparently functions as a satisfactory catalyst but is incapable of forming esters; the *trans*-triol suffers no appreciable glycol cleavage under the mild conditions required for hydrolysis (one-half hour).

Of more practical importance is the development of a reliable procedure for hydroxylating the double bond of cholesterol with hydrogen peroxide and formic acid.[6] Brief heating of cholesterol with 88% formic acid produces the 3-formyl derivative, and on addition of hydrogen peroxide to the resulting suspension a clear solution soon results and precipitation with water gives a mixture of esters from which cholestane-3β,5α,6β-triol 3,6-diformate can be isolated by crystallization. Brief saponification of the total mixture affords the pure triol in 91% yield.

Since cholestane-3β,5α-diol-6-one (III) can thus be prepared very easily in quantity from cholesterol in 85.5% over-all yield, it may serve as a useful intermediate to steroids of importance. However, two possible routes from this substance to 7-dehydrocholesterol have been investigated with negative results. The 3,5-diacetate (IV), known as a by-product of the chromic acid oxidation of cholesteryl acetate,[7] can be prepared readily by treatment of the diolone III with acetic anhydride and boron fluoride at room temperature. Since the tertiary acetoxyl group at C_5 when once formed is very resistant to saponification,[7] it seems possible that the ready acylation at this position may be the consequence of enol acetate formation and migration of the acetyl

(1) For acknowledgments, see Paper I, notes 1 and 2; THIS JOURNAL, 71, 3935 (1949).

(2) Pickard and Yates, *J. Chem. Soc.*, 93, 1678 (1908).

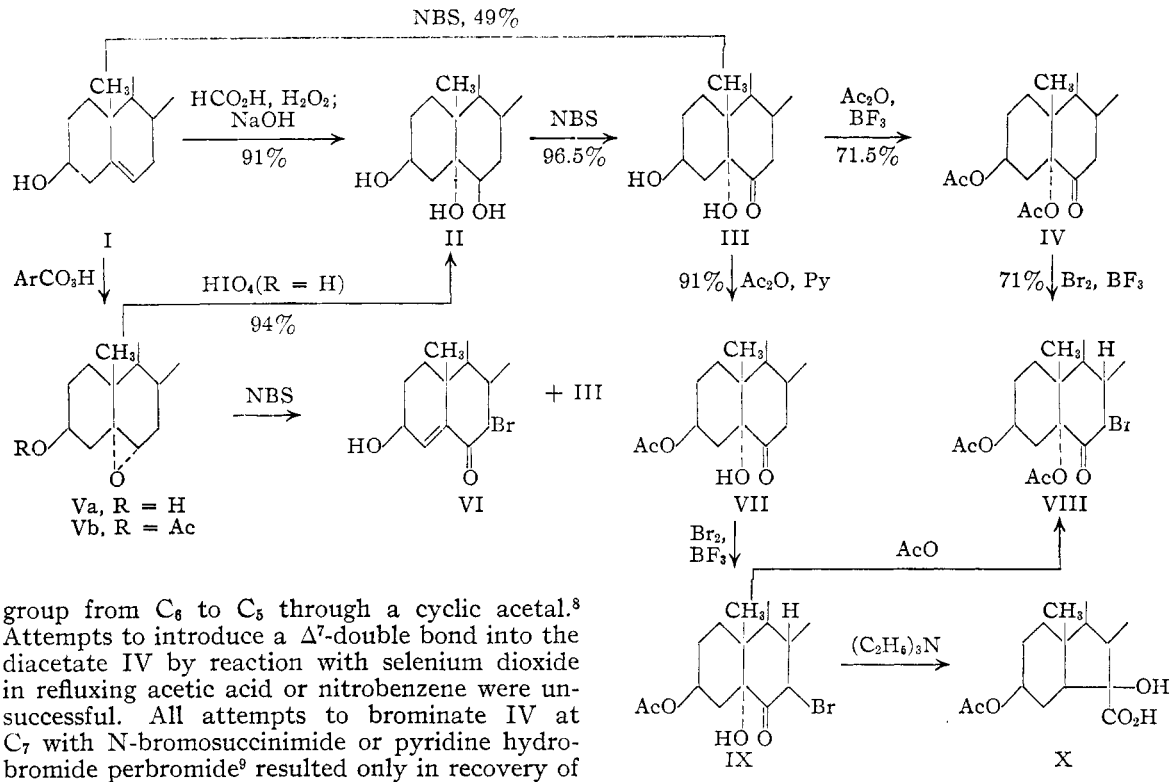
(3) Ellis and Petrow, *ibid.*, 1078 (1939).

(4) Westphalen, *Ber.*, 48, 1064 (1915); Ruzicka and Bosshard, *Helv. Chim. Acta*, 20, 244 (1937).

(5) Petrow, *J. Chem. Soc.*, 1077 (1937).

(6) Swern, Billen and Findlay, THIS JOURNAL, 67, 1786 (1945); compare Roebuck and Adkins, *ibid.*, 70, 4041 (1948); "Organic Syntheses," 28, 35 (1948).

(7) Schenck, *Z. physiol. Chem.*, 243, 119 (1936); Ellis and Petrow[3] prepared the compound from the 3-acetate with use of potassium acid sulfate as catalyst.



group from C_6 to C_5 through a cyclic acetal.[8] Attempts to introduce a Δ^7-double bond into the diacetate IV by reaction with selenium dioxide in refluxing acetic acid or nitrobenzene were unsuccessful. All attempts to brominate IV at C_7 with N-bromosuccinimide or pyridine hydrobromide perbromide[9] resulted only in recovery of starting material. Bromination finally was accomplished with use of bromine in hot acetic acid with boron fluoride as catalyst, but the chief product was contaminated with a difficultly separable isomer. More homogeneous material was obtained by similar bromination of cholestane-3β,5α-diol-6-one 3-acetate (VII), easily available by partial acetylation of the diolone (III) in pyridine. The bromo monoacetate (IX) on acetylation in the presence of boron fluoride yielded a pure diacetate (VIII) identical with the chief product of bromination of IV. This diacetate proved to be resistant to attempted dehydrobromination with triethylamine or pyridine, and hence the 7-bromo atom must have the β-configuration, *cis* to the hydrogen at C_8. The 3-monoacetate IX suffered slow dehydrobromination in refluxing triethylamine to give a mixture from which two isomers were isolated of composition corresponding to replacement of bromine by hydroxyl. The more abundant isomer, however, is acidic and probably results from a rearrangement, as in formula X. Such a rearrangement is analogous to that established[10] in the dehydrohalogenation of 5,7-dibromocholestane-3β-ol-6-one.[11] Further applications of the hydroxylation and oxidation procedures are under investigation.

(8) Compare Petrow, Rosenheim and Starling, *J. Chem. Soc.*, 135 (1943); Paige, *ibid.*, 437 (1943).

(9) Djerassi and Scholz, THIS JOURNAL, **70**, 417 (1948).

(10) Woodward and Clifford, *ibid.*, **63**, 2727 (1941).

(11) Heilbron and co-workers, *J. Chem. Soc.*, 801 (1937); 102 (1938).

Experimental[12]

Cholestane-3β,5α-diol-6-one[2] from Cholesterol (S.R.).—A suspension of 4.5 g. of cholesterol in 200 cc. of acetone and 25 cc. of water was treated with 2.5 g. (1.25 equiv.) of N-bromosuccinimide and 2.5 cc. of acetic acid and shaken occasionally at room temperature. In the course of forty-five minutes the mixture became yellow, orange, and then colorless and the solid all went into solution. After standing overnight the solution was diluted and extracted with ether, and the extract was washed with water and alkali, dried, and concentrated until crystals began separating. The material was collected and further small crops obtained by concentration of the mother liquor. Crystallization from chloroform gave colorless needles that separated in a fibrous mat and melted at 231–232°; yield 2.4 g. (49%).

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.45; H, 11.08. Found: C, 77.63, 77.75; H, 10.99, 10.76.

The identity was established by conversion to the *phenylhydrazone*,[2] m. p. 163–164°, and to the mono and diacetates as described below. When the oxidation was conducted with 3.25 equivalents of N-bromosuccinimide the yield dropped to 23%. The yield was also lower when dioxane was substituted for acetone or when no acetic acid was added. Processing of the combined ethereal mother liquors afforded a small amount of a substance that separated from ethanol in colorless needles, m. p. 122°, dec., that was identified as **5,6-dibromocholesterol** by mixed m. p. determination.

Oxidation of cholesteryl acetate (5 g.) by the same procedure gave an oil that when triturated with petroleum ether afforded 1.2 g. of crude solid, m. p. 160–170° (positive test for bromine). Several crystallizations from ligroin and methanol eventually gave a small crop of needles, m. p. 230–231°, identified as **cholestane-3β,5α-diol-6-one 3-acetate**[2] by mixed melting point comparison.

(12) Melting points are uncorrected.

Cholestane-3β,5α,6β-triol (S.R.).—A suspension of 20 g. of technical cholesterol in 200 cc. of 88% formic acid was heated to 70–80° with stirring for five minutes to form a derivative that is evidently the 3-formate, which separated as an oily layer, and cooled to 25°. The resulting thick paste of solidified formate was treated with 20 cc. of 30% hydrogen peroxide and shaken occasionally. The temperature usually rose to 35–40°; sometimes there was an even more pronounced heat effect and in this case the temperature was controlled to 40° by cooling. After about forty-five minutes the solid dissolved, the foam subsided, and a blue fluorescent solution resulted, but the temperature remained a few degrees above that of the room for about four hours longer. After a total reaction time of six to fifteen hours, the mixture was treated with 300 cc. of boiling water, stirred, allowed to cool, and the granular white solid collected, dried superficially, dissolved in 600 cc. of methanol, and the solution treated with 20 cc. of 25% sodium hydroxide, warmed on the steam-bath for ten minutes, filtered, acidified and diluted with 200 cc. of water. The white solid that precipitated was collected after cooling, washed well with water and thoroughly dried. The triol so obtained was of high purity, m. p. 236–238°, and the yield, duplicated in several experiments, was 19.7 g. (91%). Crystallization from methanol gave needles, m. p. 237–239°.

Cholestane-3β,5α,6β-triol-3,6-diformate was isolated as the chief product of the reaction of cholesterol with performic acid by crystallization of the solid material prior to saponification. Several crystallizations from methanol gave colorless needles, m. p. 180–181°, $[\alpha]^{28}D$ − 47.5° (dioxane).

Anal. Calcd. for $C_{29}H_{46}O_5$: C, 73.38; H, 9.77. Found: C, 73.54; H, 10.05.

Cholestane-3β,5α,6β-triol-3-acetate-6-formate (S.R.).—Hydroxylation of cholesteryl acetate with hydrogen peroxide in formic acid suspension as above proceeded sluggishly. There was little heat effect, and the solid did not go into solution on stirring the mixture at 40–50° for about eight hours. The precipitated product afforded triol of poor quality (m. p. 218–225°) in low yield (65%) on saponification; repeated crystallization of the unsaponified precipitate from methanol afforded colorless needles, m. p. 201–202°, $[\alpha]^{28}D$ − 43.9° (dioxane).

Anal. Calcd. for $C_{30}H_{48}O_5$: C, 73.74; H, 9.90. Found: C, 73.72; H, 9.97.

Reaction of Cholesterol α-Oxide with N-Bromosuccinimide (L.F.F.).—The α-oxide[13] was prepared by reaction of cholesterol with perphthalic acid in ether solution at room temperature. The precipitation of phthalic acid was complete in about two hours; the alkali-washed filtrate was dried, evaporated to a small volume and diluted with petroleum ether, and the α-oxide, m. p. 138–139.5°, separated in yield of 59%; recrystallized material melted at 147–148°.

A solution of 2 g. of the oxide in 80 cc. of acetone was treated with 2 cc. of acetic acid and 10 cc. of water, the precipitated material was brought into solution by gentle warming, and 1.07 g. (1.2 equiv.) of N-bromosuccinimide was added. This soon dissolved to a yellow solution and after a time long needles of the oxide separated and then, within about five hours, began to dissolve. Brief shaking resulted in a clear solution, and in a few hours a total of 0.7 g. of crystals of a different form separated. Recrystallization of this material from ethyl acetate gave large rectangular prisms, m. p. 158° dec. On further crystallization from benzene the substance, which is provisionally regarded as **7-bromo-Δ⁴-cholestene-3β-ol-6-one** (VI), formed clusters of cottony needles, m. p. 158–159°, dec., $[\alpha]^{28}D$ −33.3° (dioxane), λ_{alc}^{max} 238–243 mμ (log ε 4.2).

Anal. Calcd. for $C_{27}H_{43}O_2Br$: C, 67.62; H, 9.04. Found: C, 67.39; H, 8.84.

The mother liquor on concentration and addition of water afforded a total of 0.88 g. of material melting above 200°, but a component of constant m. p. was obtained only after several crystallizations from methanol in yield of 0.2 g. This substance formed large, flat needles, m. p. 231–232°, and gave no depression when mixed with **cholestane-3β,5α-diol-6-one.**

Cleavage of 5,6-Oxides with Periodic Acid (L.F.F.).—A solution of 1 g. of cholesterol α-oxide in 30 cc. of hot acetone was treated with a solution of 0.625 g. of periodic acid dihydrate in 10 cc. of water. Before all of the precipitated oxide had redissolved, thin plates of the cleavage product began to separate. The mixture was refluxed for one-half hour, cooled, and the product collected and washed with acetone–water (1:1). The thoroughly dried material (0.83 g.) melted at 231–232° and showed no depression when mixed with cholestane-3β,5α,6β-triol. Dilution of the mother liquor at the boiling point afforded a second crop of 0.14 g. of crystals, m. p. 225–226° (total yield, 94%). Recrystallization of the combined crops from methanol afforded flat needles, m. p. 234–235°.

Cholesteryl α,β-oxide acetate (8 g.) was refluxed for one hour in acetone solution (140 cc.) with 2 g. of periodic acid dihydrate in 20 cc. of water and the solution was filtered and concentrated to the point of saturation. A first crop of cholestane-3β,5α,6β-triol-3-acetate, m. p. 205–206°, amounted to 5.93 g. Concentration of the mother liquor afforded three further crops that separated either as plates or prisms and melted in the range 203–206°. The total yield of 3-acetate was 7.24 g. (87%).

Cholestane-3β,5α-diol-6-one (III) from the *trans*-Triol (S.R. and L.F.F.).—The selective oxidation of the triol at C_6 by N-bromosuccinimide proceeds readily in aqueous acetone or methanol with either a small or large excess of reagent. The following procedures were found particularly satisfactory.

(a) **In Aqueous Dioxane.**—A solution of 10 g. of triol in 90 cc. of dioxane was diluted with 10 cc. of water, cooled to 25° and treated with 4.5 g. (1.05 equiv.) of N-bromosuccinimide, which promptly dissolved. In the course of three to four minutes the color changed to yellow, deep orange, light yellow, and colorless, and the reaction product began to separate. The temperature was kept at 25° by cooling, and after ten minutes the mixture was cooled in ice and the diolone collected and washed with 50% methanol; the fully dried material weighed 6.7 g., m. p. 232–233°, dec. The mother liquor was diluted with water and extracted with ether, and the washed and dried solution was concentrated until crystals of the diolone began to separate, and a further crop of 2.5 g. of ketone of satisfactory purity was obtained; total yield of material, m. p. 231–233°, dec., 9.2 g. (93%).

In an experiment conducted without the addition of water the reaction was slow and the reaction product was obtained in low yield and very inferior quality.

(b) **In Aqueous Methanol–Ether.**—A 1-liter separatory funnel was charged with 23 g. of the triol, 450 cc. of ether, 75 cc. of methanol, 75 cc. of water and 10.8 g. (1.05 equiv.) of N-bromosuccinimide and shaken to effect solution. Oxidation was over in a few minutes and gave an orange-yellow solution. On addition of water the color became lighter and the bulk of the diolone separated from the organic phase as colorless, shiny needles. The water phase was tapped off and the suspension in ether washed with bisulfite solution, with alkali and with water. The ketone was then collected on a Buchner funnel and washed with ether to give a first crop of 19 g., m. p. 232–233°, dec. Successive concentrations of the mother liquor afforded two additional crops amounting to 3 g., m. p. 232–233°, dec.; total yield 22 g. (96.5%).

In parallel experiments on one-tenth the above scale with 1.05 and with 2.1 equivalents of N-bromosuccinimide, the yield of product in the first crop was 1.94 g. (m. p. 231–232°) and 1.91 g. (m. p. 231–232°), respectively.

Cholestane-3β,5α-diol-6-one 3-Acetate.[3] **(a) By Acetylation (S.R.).**—A mixture of 10 g. of the diolone, 50 cc. of acetic anhydride and seven drops of pyridine was heated to the boiling point, allowed to cool to room temperature and treated with water. The reaction product consisting

(13) Hattori, *J. Pharm. Soc., Japan*, **60**, 334 (1940) (*C. A.*, 7294 (1940)).

of colorless needles was washed with a little methanol and dried; yield 10.7 g. (91%). Material recrystallized from methanol melted at 232–233° and did not depress the sample (b).

(b) **By Oxidation (L.F.F.).**—A mixture of 400 mg. of cholestane-3β,5α,6β-triol 3-acetate and 200 mg. (1.3 equiv.) of N-bromosuccinimide was dissolved in 15 cc. of acetone by slight warming, cooled to 25° and treated with 1 cc. of water. The solution turned pale yellow in one or two minutes, crystals began to separate in ten minutes and the yellow color disappeared in twenty-five minutes. After a total of one and one-half hours 10 cc. of water was added and the precipitated solid collected; yield 370 mg. (93%), m. p. 229–230°; recrystallized: m. p. 232–233°.

Cholestane-3β,5α-diol-6-one 3,5-Diacetate[2,3] **(L.F.F. and S.R.).**—A suspension of 10 g. of the diolone in 50 cc. of acetic anhydride was treated with six drops of boron fluoride etherate, stirred, and the lumps broken up with a stirring rod. The bulk of the solid rapidly dissolved with a slight rise in temperature, and complete solution was effected by brief warming on the steam-bath. After fifteen minutes water was added and the crystalline yellow solid that soon separated was collected and the color removed by washing with a little methanol. Crystallization from methanol afforded 8.5 g. (71.5%) of the diacetate as prismatic needles, m. p. 170–171°.

Anal. Calcd. for $C_{31}H_{50}O_5$: C, 74.04; H, 10.02. Found: C, 74.25; H, 10.14.

A sample of the diacetate (5 g.) was refluxed with selenium dioxide (2.5 g.) in acetic acid (50 cc.) for six hours, but was recovered unchanged (4.7 g.). A similar experiment conducted in refluxing nitrobenzene (2 g. diacetate, seven hours) led to extensive tar formation, and the only product isolated was starting material (0.8 g., m. p. 169–170°).

7β-Bromocholestane-3β,5α-diol-6-one 3-acetate (L.F.F.).—A solution of 3.23 g. of cholestane-3β,5α-diol-6-one 3-acetate in 70 cc. of acetic acid was treated at 60° with a solution of 1.23 g. of bromine in 35 cc. of acetic acid and 1 cc. of boron fluoride etherate, kept at 60° for fifteen minutes, when the solution had become light yellow, let stand for one hour and diluted with 100 cc. of water. The dried precipitate (3.71 g., m. p. 88–100°) was ground in a mortar with a little methanol, when initially a part dissolved and a part formed a gum. Further trituration produced a thick paste of white solid, which when collected, washed and dried weighed 2.90 g. (71%).

The bromo derivative is very readily soluble in ether, fairly soluble in petroleum ether and moderately soluble in hot methanol (about 30 cc./g.). On repeated crystallization from methanol it formed a cottony mat of fine needles of the constant m. p. 170–171°; $[\alpha]^{28}D$ +7.5° (dioxane).

Anal. Calcd. for $C_{29}H_{47}O_4Br$: C, 64.55; H, 8.78. Found: C, 64.60; H, 8.63.

Dehydrobromination was accomplished by refluxing a solution of 1 g. of the 3-acetate in 10 cc. of triethylamine. The amine hydrobromide is insoluble in the boiling amine; the amount collected after seven hours and washed with ether represented 28% of the theory, and after twenty-four hours of refluxing the total yield of salt was 267 mg. (93%). The only slightly discolored filtrate and washing were evaporated at reduced pressure and the residue was washed in ether with acid and water and the solution dried and evaporated. The resulting light brown gum was chromatographed in petroleum ether (30–60°) by Dr. Huang-Minlon. Elution with petroleum ether–benzene (1:3; 1:1; 2:1) and with pure benzene gave fourteen fractions, the first five of which were oily. The next three fractions melted in the range 150–172° and were halogen-free, and crystallization of the total from acetone–ligroin (70–90°) afforded a few milligrams of isomer A, m. p. 173–175°.

Fraction 11 afforded 125 mg. of halogen-free crystals, m. p. 167–168°. Recrystallization from methanol (m. p. 168–169°) and then from acetone–petroleum ether gave isomer B (probably X), m. p. 170–171°. The substance is only slightly soluble in boiling dilute soda solution, but the cooled and filtered solution gives a definite precipitate on acidification.

Anal. Calcd. for $C_{29}H_{48}O_5$: C, 72.97; H, 10.19. Found, A: C, 73.36, 73.28; H, 10.00, 10.15. B: C, 73.24; H, 10.30.

7β-Bromocholestane-3β,5α-diol-6-one 3,5-Diacetate (L.F.F.).—A solution of 1.5 g. of the 7β-bromo 3-acetate in 13 cc. of hot acetic anhydride was treated with five drops of boron fluoride etherate and let cool, when 1.5 g. (79.5%) of the diacetate separated in crystalline form, m. p. 214–216°. Recrystallization from 250 cc. of methanol afforded 1.29 g. of needles, m. p. 216.5–217.5°, $[\alpha]^{25}D$ +37.0° (dioxane).

Anal. Calcd. for $C_{31}H_{49}O_5Br$: C, 64.01, H, 8.49. Found: C, 64.09; H, 8.42.

Attempts to brominate the diolone 3,5-diacetate with N-bromosuccinimide with and without irradiation and peroxide resulted only in recovery of unchanged starting material. Bromination of the diacetate (503 mg.) in acetic acid (5 cc.) with a solution of bromine (0.176 g.) in acetic acid (5 cc.) was effected by adding boron fluoride etherate (6 drops) and warming the solution to 75° for twenty-five minutes, when the color had faded to light yellow. On addition of 3 cc. of water and cooling a solid product was obtained; 450 mg., m. p. 201–203°, dec. One crystallization from 45 cc. of methanol gave long spars, m. p. 205–207°, dec. (325 mg.), and further purification gave material of the expected composition; m. p. 210–211°, dec., $[\alpha]^{25}D$ +27° (dioxane).

Anal. Calcd. for $C_{31}H_{49}O_5Br$: C, 64.01; H, 8.49. Found: C, 63.74; H, 8.44.

This material apparently consists chiefly of the above 7β-bromo diacetate containing a small amount of a less dextrorotatory isomer (7α-epimer ?), but several further crystallizations raised the m. p. only to 213–214°; a mixture of this with the 216.5–217.5° material melted at 214–215°.

The 216.5–217.5° diacetate (1.3 g.) was refluxed with triethylamine (80 cc.) for fifty-eight hours, but only about 100 mg. of triethylamine hydrobromide separated and the only product encountered was starting material (0.6 g.). The 210–211° product was recovered unchanged after being boiled with pyridine for six hours; this was also true when silver benzoate was added, but the recovery was in this case lower.

Summary

1. A procedure for the hydroxylation of the double bond of cholesterol by brief treatment with performic acid and saponification affords cholestane-3β,5α,6β-triol in 91% yield.

2. The above triol can be selectively oxidized to the 6-ketone with N-bromosuccinimide in 94% yield, even in the presence of methanol.

3. The 5,6-oxido derivatives of cholesterol and cholesteryl acetate can be cleaved to the glycols by reaction with periodic acid in acetone.

4. Bromination of the mono- or diacetate of cholestane-3β,5α-diol-6-one was accomplished with use of boron fluoride catalyst, but the chief products are evidently the 7β-bromo derivatives, since that from the diacetate resists dehydrohalogenation and that from the 3-monoacetate yields an acidic product of rearrangement.

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