One method consisted in the bromination of Δ^5-unsaturated steroids with N-bromosuccinimide followed by catalytic replacement of the 7-bromo group of the product with deuterium. The deuterium atom in 7-*d*-Δ^5-steroids prepared in this manner was shown to be stably bound. The other procedure involved the desulfurization of suitable steroid mercaptols with "deuterized" Raney nickel affording d_2-steroids. The mercaptols used in this study were readily prepared from Δ^5-7-ketosteroids by condensation with ethanedithiol in the presence of anhydrous zinc chloride. The ultraviolet spectra of the ethylene mercaptols have been determined and the contribution of the dithiolane ring estimated.

NEW YORK 21, N. Y. RECEIVED MAY 1, 1950

[FROM THE DIVISION OF STEROID BIOCHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Δ^5-Cholestene-3β,4β,7α-triol and the Inhibition of the Oxidation of Hydroxyl Groups by Vicinal Substituents

BY SEYMOUR LIEBERMAN AND DAVID K. FUKUSHIMA

In the preceding paper[1] we mentioned the isolation of 3β-acetoxy-Δ^5-cholestene-4β,7α-diol (II), m. p. 175–176°, from the reaction of cholesterol acetate (I) with excess N-bromosuccinimide. The evidence for this structure is presented in this paper and is based upon a study of degradation products, molecular rotation differences, and ultimately conversion to a known compound whose constitution is well established. 3β-Acetoxy-Δ^5-cholestene-4β,7α-diol (II) exhibited certain unexpected properties toward oxidizing agents, and since these properties are of some interest in the field of neighboring group reactions, we have extended our studies to encompass other instances of these effects.

The analysis of II satisfied the empirical formula $C_{29}H_{48}O_4$ and the method of preparation involving the use of an excess of N-bromosuccinimide suggested that the product was a 3β-acetoxy-Δ^5-cholestene-4,7-diol derived from cholesterol acetate by α,α' dibromination[2,3,4] followed by replacement of the allylic bromide by hydroxyl during chromatography.[1,5,6] The compound formed a triacetate (IIIb), $C_{33}H_{52}O_6$, m. p. 172–173.5°, $[\alpha]_D$ −175°, and was saponified to a triol, $C_{27}H_{46}O_3$ (IIIa), m. p. 195–197°, $[\alpha]_D$ −102°.

A search of the literature for derivatives of Δ^5-cholestenetriol-3,4,7 revealed that in 1946 Petrow and Starling[7] reported the preparation of two isomers, Δ^5-cholestenetriol-3β,4β,7β[8] and Δ^5-cholestenetriol-3β,4β,7α[8] whose physical constants and those of their tribenzoates are listed in Table I. A comparison of these data with the physical constants of Compound IIIa led us to believe that it was not identical with either of the isomeric triols; the tribenzoate IIIc appeared to confirm this conclusion because it differed significantly from either tribenzoate.

There was a possibility that II was not the 4,7-dihydroxy compound but was a rearrangement

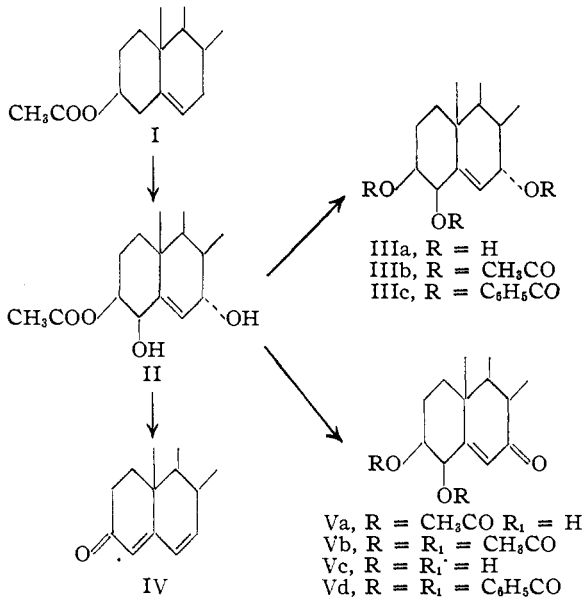


Fig. 1.

TABLE I

PHYSICAL CONSTANTS OF Δ^5-CHOLESTENE-3β,4β,7-TRIOLS AND THEIR TRIBENZOATES

Δ^5-Cholestenetriol-	M. p., °C.	$[\alpha]_D$	Ref.
3β,4β,7β	188–190	+ 5.0°	7
Tribenzoate	190–192	+ 99.6°	7
3β,4β,7α	softens 130, 169–170	− 96.9°	7
Tribenzoate	150–152	− 45.0°	7
Triol IIIa	195–197	−102.0°	
Tribenzoate IIIc	159–160	− 79.7°	

(1) Fukushima, Lieberman and Praetz, THIS JOURNAL, **72**, 5205 (1950).

(2) Ziegler, Späth, Schaaf, Schumann and Winkelmann, *Ann.*, **551**, 80 (1942).

(3) Howton, THIS JOURNAL, **69**, 2060 (1947).

(4) Barnes, *ibid.*, **70**, 145 (1948).

(5) Buisman, Stevens and v. d. Vliet, *Rec. trav. chim.*, **66**, 83 (1947).

(6) Sutton and Dutta, *J. Chem. Soc.*, 939 (1949).

(7) Petrow and Starling, *ibid.*, 749 (1946).

(8) Petrow and Starling[7] assigned the opposite configuration to the C7-OH groups. The configurations used throughout this paper are those of Shoppee (*Annual Reports*, **43**, 200 (1947)), who pointed out that these designations are in better agreement with those predicted by molecular rotatory data.

product resulting from an allylic shift of one of the hydroxyl groups. If this were true, the two possible rearrangement products would be either 3β-acetoxy-Δ⁶-cholestene-4,5-diol or 3β-acetoxy-Δ⁴-cholestene-6,7-diol. These 1,2-glycols were eliminated from consideration when it was found that II was not oxidized by periodic acid, and therefore its two unacetylated hydroxyl groups were not vicinal. The formation of a triacetoxy derivative (IIIb) from II by acetylation at room temperature was added evidence that II could not be the 4,5-diol since both hydroxyl groups appeared to be secondary. On the other hand, the triol IIIa, obtained by saponification of II, was oxidized by periodic acid, revealing that one of the two hydroxyl groups in II was adjacent to the 3-acetoxy group. That rearrangement during saponification of II had not occurred was shown by the fact that the same triacetate IIIb could be obtained by acetylation from both the monoacetate (II) and the triol (IIIa). The dialdehyde resulting from the periodate oxidation of the triol exhibited an absorption maximum at 228 mμ, and its disemicarbazone exhibited maxima at 232 and 267 mμ, clear evidence that one aldehyde group was conjugated with a double bond.[9] This suggested that the hydroxyl group which was shown to be vicinal to the 3-acetoxy group was also alpha to the double bond and therefore was at C_4.

Several attempts were made to oxidize II to the corresponding 3β-acetoxy-Δ⁵-cholestene-4,7-dione because the ultraviolet absorption characteristics of this enedione (maximum at 252 mμ) would unequivocally relate the two new functional groups to each other. Three methods of oxidation were used, and none gave the expected product. Oppenauer oxidation with aluminum tertiary butylate and cyclohexanone converted II into Δ⁴,⁶-cholestadien-3-one (IV). The formation of this product could be explained by assuming that dehydration rather than oxidation of the free hydroxyl groups had occurred, resulting in one of several possible unsaturated 3-enolacetates (as, for example, Δ³,⁵,⁷-cholestatrien-3β-ol acetate). These, on saponification of the 3-acetoxyl group either during the treatment with aluminum tertiary butylate or during the subsequent workup of the reaction mixture, can ketonize to yield 6-dehydrocholesten-3-one. A somewhat similar observation was made by Wintersteiner and Ruigh,[10] who isolated 6-dehydrocholesten-3-one as a side product from the Meerwein–Ponndorf reduction of 7-ketocholesterol acetate. They postulated that the carbonyl group at C_7 was reduced and the resulting hydroxyl group dehydrated. Saponification of the 3-acetoxy group and subsequent oxidation resulted in the formation of 6-dehydrocholesten-3-one. That dehydration[11,12,13] and saponification of 3-acetoxy groups[14] can occur during reaction with aluminum alkoxides has been reported previously. The formation of 6-dehydrocholesten-3-one from II was therefore additional evidence that the hydroxyl groups were at C_4 and C_7.

Oxidation of the monoacetate, II, with chromic acid in acetic acid resulted in the oxidation of only one of the two secondary hydroxyl groups. The product (Va) exhibited an absorption maximum at 235 mμ characteristic of an α,β-unsaturated ketone rather than at 252 mμ as expected for an enedione. That the unoxidized hydroxyl group was secondary and not tertiary was shown by acetylation of Va to the diacetate, Vb, $C_{31}H_{48}O_5$, $\log \epsilon_{231\ m\mu} = 3.97$. When II was oxidized with N-bromoacetamide, the same α,β-unsaturated ketone, Va, was obtained. Mild saponification of Va gave the α,β-unsaturated dihydroxy ketone Vc, m. p. 199–205°; $[\alpha]_D$ −60.6°, absorption maximum at 234.5 mμ. Although the physical constants of this substance agreed well with the 7-keto-Δ⁵-cholestene-3β,4β-diol (m. p. 205–206°; $[\alpha]_D$ −71.4°; $\log \epsilon_{238\ m\mu}$ 3.96) reported by Petrow and Starling,[7] no attempt was made to purify it further, because only 16 mg. of this difficultly crystallizable substance was available. Vc was benzoylated in pyridine solution, since Petrow and Starling reported that the dibenzoate of 7-keto-Δ⁵-cholestene-3β,4β-diol (Vd) crystallized well. The product obtained after careful chromatographic analysis and recrystallization from ethanol melted at 144–144.5°; $[\alpha]_D$ −47.4° ±4°; $\log \epsilon_{232\ m\mu}$ 4.57 and agreed with the constants reported by Petrow and Starling (m. p. 145–146°; $[\alpha]_D$ −45.5°).

The identity of Compound Vd with Petrow and Starling's dibenzoate of 7-keto-Δ⁵-cholestene-3β,4β-diol enabled us to draw the following conclusions: (1) that Compound II does, indeed, have its unacetylated hydroxyl groups on C_4 and C_7; (2) that the C_4-OH of II and V is β oriented; and (3) that it was the OH on C_7 in II which was oxidized preferentially with chromic acid or with N-bromoacetamide. These facts prove that IIIa must be one of the two C_7 isomers of Δ⁵-cholestene-3β,4β,7-triol in spite of the fact that its melting point and rotation did not correspond with the isomers prepared by Petrow and Starling. This difficulty was easily resolved, however, by assuming that IIIa was the 7α isomer and that the compound assigned that structure by Petrow and Starling was contaminated with the 7β isomer. This is reasonable since the two C_7 epimeric triols obtained by these investigators were separated from each other after the Meerwein–Ponndorf reduction of the corresponding 7-ketone. Thus, if Petrow and Starling's 3β,4β,7α-triol was contaminated with some of the 7β isomer, its melting point would be lowered, but its optical rotation (−96.9°) might be expected to be close to

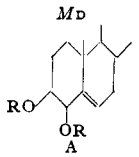
(9) Evans and Gillam, *J. Chem. Soc.*, 565 (1943).
(10) Wintersteiner and Ruigh, THIS JOURNAL, **64**, 2453 (1942).
(11) Henbest and Jones, *J. Chem. Soc.*, 1792 (1948).
(12) Ruzicka and Muhr, *Helv. Chim. Acta*, **27**, 503 (1944).
(13) Marker and Turner, THIS JOURNAL, **64**, 481 (1942).
(14) Windaus and Schenck, U. S. Patent 2,098,985 (1937).

that of IIIa (−102°) since the rotation of the contaminant (+5.0°) would make only a small contribution. Similarly, if their 3β,4β,7α-tribenzoate were contaminated with the 7β isomer, its optical rotation (−45.0°) would be considerably lower than that of IIIc (−79.7°) since even a small amount of contaminant (+99.6°) would significantly alter the rotation.

Further confirmation that IIIa is the 3β,4β,7α-isomer can be acquired from a consideration of molecular rotatory data.[15,16] The observed M_D of Compound IIIa and its triacetate and tribenzoate (Table II) are in complete accord with those calculated for Δ^5-cholestene-3β,4β,7α-triol derivatives from independent data.

TABLE II

THE COMPARISON OF THE CALCULATED AND OBSERVED MOLECULAR ROTATIONS OF Δ^5-CHOLESTENE-3β,4β,7-TRIOLS AND THEIR ESTERS



	M_D		Rotatory contributions[a] ΔM_D (C_7–OR)	M_D Calcd. A + B	M_D Observed
R = H	−242	7α	−194	− 436	−427
					−405[b]
		7β	+183	− 58	+ 21[b]
R = Bz	−330	7α	−583	− 913	−584
					−329[b]
		7β	+648	+ 318	+728[b]
R = Ac	−468	7α	−640	−1108	−955
		7β	+434	− 34	

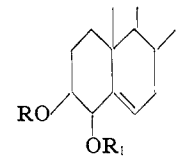
[a] ROTATORY EFFECTS.—These are calculated by subtracting the M_D of the 3-substituted derivatives of Δ^5-cholestene from the M_D of the 3,7-disubstituted derivatives of Δ^5-cholestene, *e. g.*, M_D (Δ^5-cholestene-3β,7α-diol) − M_D (Δ^5-cholesten-3β-ol) = ΔM_D (7α-OH). Barton and Cox (*J. Chem. Soc.*, 783 (1948)] have emphasized the serious anomalies which are introduced into calculations based on molecular rotatory differences by the interaction or influence of neighboring, polarizable, or distorting groups. This is especially true in the case of Compound III where the four functional groups are aligned on successive carbon atoms. To minimize as much as possible the effects of vicinal action, the rotatory data from closely analogous compounds were used. [b] Data of Petrow and Starling.[7]

It is important to point out that no rigorous proof has been given for the formulation of II as the 3-monoacetate of the triol. Because 3β-acetoxy-4β-hydroxy-Δ^5-steroids undergo acyl migration (*vide infra*), the possibility exists that II is the isomeric 4β-acetoxy-Δ^1-cholestene-3β,7α-diol. The evidence accumulated establishes the structure of the triol IIIa and its derivatives even though it does not fix definitely the position of the acyl group of the monoacetate II.

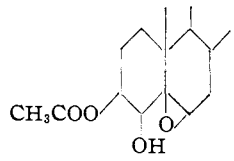
NOTE ADDED IN PROOF.—Recent evidence obtained in this laboratory by Mr. Robert W. Jailer indicates that II is 4β-acetoxy-Δ^5-cholestene-3β,7α-diol, demonstrating that acyl migration had indeed occurred during chromatography. The full details will be published shortly.

The Inhibition of the Oxidation of Hydroxyl Groups by Vicinal Substituents.—The fact that the C_4-OH group of Compound II was not oxidized by N-bromoacetamide[17] or by chromic acid is interesting because it is undoubtedly related to the presence of the vicinal *cis*-acetoxyl group on C_3 and therefore is an example of the role of neighboring groups in determining the course of reaction. Winstein and his associates[18] have extensively studied the role of the acetoxyl group on the stereochemical course of replacement reactions of adjacent groups.

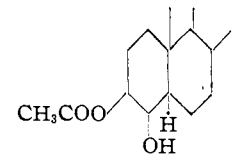
In order to study the effect of neighboring groups on the course of oxidation, a number of steroids structurally similar to Compound II has been investigated. As in the case of II, the 3β-acetoxyl group present in 3β-acetoxy-Δ^5-cholesten-4β-ol (VIa) appeared to inhibit the oxidation of the 4β-OH group by N-bromoacetamide when the reaction was carried out in either pyridine[19] or



VIa R = CH_3CO R_1 = H
VIb R = C_6H_5CO R_1 = H
VIc R = R_1 = CH_3CO
VId R = R_1 = CH_3CO



VII



VIII

Fig. 2.

t-butanol at room temperature for twenty-one hours. The products obtained from these experiments exhibited no absorption in the ultraviolet characteristic of an α,β-unsaturated ketone, the expected product of oxidation. Approximately 85% of the material was recovered either as the 3β-acetoxy compound (VIa) or the 4β-acetoxy compound (VIc) which resulted from VIa by acyl migration on the alumina used for chromatog-

(15) Barton and Klyne, *Chemistry and Industry*, 755 (1948).

(16) Fieser and Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Corp., New York, N. Y., 1949, p. 206.

(17) Another example of the resistance to oxidation of some secondary hydroxyl groups in the steroid nucleus has recently been described by Fieser and Rajagopalan (THIS JOURNAL, **71**, 3935 (1949)), who have shown that N-bromosuccinimide selectively oxidizes the $C_7\alpha$–OH group but is completely unreactive toward the $C_{12}\alpha$–OH and $C_{12}\alpha$–OH groups. In contrast, N-bromoacetamide oxidized all three hydroxyl groups.

(18) Winstein, *et al.*, THIS JOURNAL, **64**, 2780, 2787, 2796 (1942); **65**, 613 (1943); **70**, 812 (1948).

(19) The N-bromoacetamide oxidation of these steroids was carried out in pyridine instead of *t*-butanol (Reich and Reichstein, *Helv. Chim. Acta*, **26**, 562 (1943)), since some steroids were only slightly soluble in the latter solvent. In pyridine solution cholestan-3β-ol was oxidized by N-bromoacetamide to cholestan-3-one in 93% yield in seven hours.

raphy.[20] Even after sixty hours, no oxidation by N-bromoacetamide was observed. When the acyloxy group on C_3 was benzoxy (VIb), the result was similar; the unoxidized benzoate was recovered in 83% yield. As Petrow and Starling have already shown,[21] when VIa was oxidized with chromic acid in glacial acetic acid, the product was the 5,6-epoxide of the 3β-acetoxy-Δ⁵-cholesten-4-one, an unusual example of an epoxidation of a *t–s* double bond. Similarly, when there was a free hydroxyl group at C_3 and an acetoxyl group on C_4 as in compound VIc, the oxidation of the unacetylated hydroxyl group by N-bromoacetamide did not take place. The resistance to oxidation of a free hydroxyl group is thus dependent on the proximity of the neighboring group and not on the position of the hydroxyl group in the steroid nucleus.

Whether a neighboring acetoxyl group *trans* to the hydroxyl would also inhibit the oxidation by N-bromoacetamide was not determined, because we were unable to prepare the model substance, 3β-acetoxy-Δ⁵-cholesten-4α-ol. In marked contrast to the relative ease with which the 3-monoacetate (VIa) of the *cis* diol was formed by partial acetylation, the monoacetate of the *trans* diol could not be obtained by this method. Both hydroxyl groups appeared to be acetylated simultaneously even when approximately one mole of acetic anhydride was used.

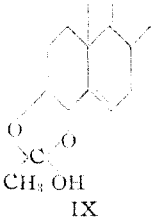
The compounds studied above, as well as the monoacetate II, were Δ⁵-unsaturated steroids. To determine the effect of the double bond on the anomalous behavior of these hydroxyl groups, a comparison was made with the corresponding 5,6-epoxy-3,4-diol, as well as with the corresponding saturated 3,4-diol. The 5,6-epoxy-3β-acetoxycholestan-4β-ol (VII) was easily oxidized by chromic acid to the 4-keto-epoxide, 5,6-epoxy-3β-acetoxycholestan-4-one, identical with the product obtained directly from VIa. VII was not oxidized by N-bromoacetamide, evidence that the vicinal groups exerted a similar influence to that which has been shown for the Δ⁵-diols. The saturated 3β-acetoxycholestan-4β-ol (VIII), on the other hand, was oxidized by N-bromoacetamide. The rate of oxidation was, however, markedly slower than usual for a secondary alcohol.[19] After twenty-one hours at room temperature 44% of the 3β-acetoxycholestan-4-one and about 30% of the starting material were obtained. Even after sixty-four hours at room temperature only 73% of the ketone was isolated. This difference in rate offered strong support for the view that the vicinal effect was operative in this system, even though to a lesser degree than in the completely resistant Δ⁵-3,4-diol monoacetates.

(20) Petrow, Rosenheim and Starling (*J. Chem. Soc.*, 135 (1943)) and Paige (*ibid.*, 437 (1943)) explain the acyl migration which occurs when the 3-monoacetate of Δ⁵-cholestene-3β,4β-diol (VIa) is heated in acetic acid to yield a 4-monoacetate (VIc) by assuming the formation of an intermediate cyclic orthomonoacetate.

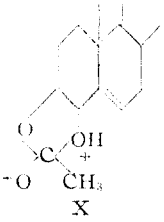
(21) Petrow and Starling, *ibid.*, 60 (1940)

These results demonstrate that vicinal groups play an important role in oxidative as well as in the replacement reactions examined by Winstein and his associates.[18] In the series of compounds we have examined, the saturated compound (VIII) is least resistant to oxidation. It is oxidized by both chromic acid and N-bromoacetamide, although at a slower rate by the latter oxidant than is usual for a secondary hydroxyl group. The unsaturated compound (VIa) and its epoxide (VII) are next in order, being entirely resistant to N-bromoacetamide but not to chromic acid. The compound exhibiting the greatest resistance to oxidation is Va, which in addition to all the structural features of VIa has a carbonyl group in conjugation with the Δ⁵ double bond. This substance is oxidized by neither N-bromoacetamide nor chromic acid. Thus, while a vicinal 3β-acetoxy group impedes the oxidation of the 4β-hydroxyl group, an additional participating group (double bond, epoxide, or α,β-unsaturated carbonyl) enhances this effect. It is important to note that the most polar group, the α,β-unsaturated carbonyl, has the greatest influence on the inhibition.

A number of factors such as hydrogen bonding, steric hindrance, and intermediary cyclic orthomonoacetate formation come to mind as possible causes for this phenomenon. On closer scrutiny, however, it is apparent that none of these satisfactorily accounts for all the observations. Infrared spectral analysis of these compounds[22] has failed to reveal any evidence of strong hydrogen bonding in spite of the fact that the spectra were determined in chloroform or carbon tetrachloride, non-polar solvents in which the hydrogen bonding should have been clearly apparent. If the formation of ortho acetates such as IX were responsible for the inhibition, one would expect the products recovered from the action of N-bromoacetamide on 3β-acetoxy-Δ⁵-cholesten-4β-ol (VIa) and on 4β-acetoxy-Δ⁵-cholesten-3β-ol (VIc) to be identical, since both compounds would give rise to the same intermediate, but this was not the case.



IX



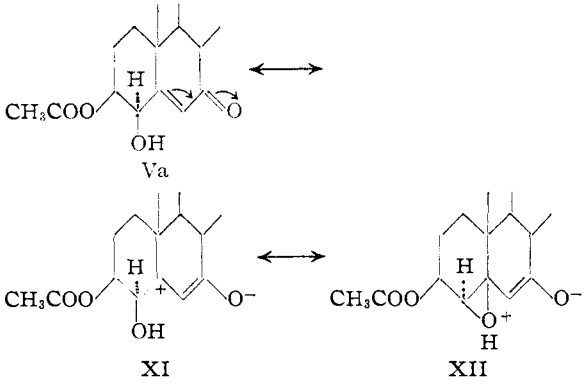
X

Furthermore, there were no significant distortions in the acetoxyl absorptions at about 1740 cm.$^{-1}$ and about 1240 cm.$^{-1}$ of these compounds to suggest the existence of an ortho acetate, at least in carbon disulfide, the solvent in which the spectra

(22) We are greatly indebted to Dr. R. N. Jones of the National Research Council of Canada and Dr. K. Dobriner of this Institute for these infrared determinations. A lithium fluoride prism was used in order to increase as much as possible the dispersion in the O–H region (3600 cm.$^{-1}$).

were determined. Examination of Fisher–Hirschfelder–Taylor atom models does not reveal any steric hindrance due to the bulk of the neighboring substituents. The role of the solvent in determining the course of these oxidations is another factor that must be considered.

If the mechanism of oxidation of a secondary hydroxyl group is assumed to be initiated by the attack of the oxidant on the hydrogen attached to the carbon bearing the hydroxyl group, followed by the removal of hydrogen as hydride ion,[23] then any factor which results in the shifting of the electrons closer to that carbon would inhibit the removal of the hydride ion. Such a situation may exist in an oxonium ion, X, which is one of the resonance forms of Compound VIa. The electrons of the C_4–H bond are attracted to the carbon by the positive charge so that the hydrogen is less easily removed. The contribution of such a system may not be sufficient to inhibit the oxidation to any extent, but the addition of another vicinal polar group appears to enhance this effect. Thus, in Compound Va, the polarization of the α,β-unsaturated carbonyl group could give rise to ionic resonance forms such as XI and XII which results in drawing the electrons of the C_4-OH bond closer to the carbon. This adds to and



enhances the inhibitory effect of the neighboring acetoxyl group, so that the C_4–OH in Va is not oxidized even with chromic acid. In a similar manner, the 5,6-double bond and the 5,6-epoxy group in Compounds VIa and VII also enhance the inhibitory effect of the neighboring acetoxyl group so that the C_4–OH is not oxidized by N-bromoacetamide. However, the contribution of these groups is not sufficient to inhibit the oxidation by chromic acid. This effect of the polar groups can also be exerted on C_3 in Compound VIc by the *i* mechanism.

Acknowledgment.—The authors gratefully acknowledge the assistance of the Jane Coffin Childs Memorial Fund for Medical Research, the Commonwealth Fund, the Lillia Babbit Hyde Foundation, the National Cancer Institute of the United States Public Health Service, the American Cancer Society, and the Teagle Fellowship Fund. We are also indebted to Bernadette Praetz and Jean Rogers for their excellent technical assistance. We wish also to express our deep appreciation to Dr. T. F. Gallagher for his assistance in the preparation of this manuscript.

(23) Bartlett, *Record of Chemical Progress*, **11**, 47 (1950); Westheimer and Nicolaides, THIS JOURNAL, **71**, 25 (1949).

Experimental[24]

3β-Acetoxy-Δ^5-cholestene-4β,7α-diol (II).—A mixture of 5.0 g. of cholesterol acetate and 5.0 g. of N-bromosuccinimide in 65 cc. of carbon tetrachloride was refluxed for twenty minutes. The mixture was then cooled and the precipitated succinimide removed by filtration. The carbon tetrachloride filtrate was extracted successively with water, sodium bisulfite solution and water. It was dried over sodium sulfate and the solvent removed *in vacuo* at 40°. The residual brown oil could not be crystallized and was therefore chromatographed on 100 g. of magnesium silicate–Celite (1:1) mixture. The oil was dissolved in 300 cc. of carbon tetrachloride, and the solution was poured onto the adsorbent. The column was developed with carbon tetrachloride (1200 cc.), carbon tetrachloride:benzene (1:3) (2100 cc.), carbon tetrachloride:benzene (1:1) (1500 cc.), benzene (900 cc.), benzene solution containing 5% ether (2100 cc.), ether (1800 cc.), ether containing 5% acetone (300 cc.), ether containing 10% acetone (300 cc.), ether containing 25% acetone (300 cc.), acetone (600 cc.), and methanol (900 cc.). The fractions eluted with these solvents were all oils, and only the reddish-orange oil (2.26 g.) obtained from the ether and acetone–ether eluates crystallized from methanol in the cold. Further recrystallization from methanol, acetone, and then ether gave 620 mg. of 3β-acetoxy-Δ^5-cholestene-4β,7α-diol (II), m. p. 174.5–176°; $[\alpha]^{30}D$ −106 ± 2° (10.39 mg. in 2.0 ml. of chloroform). Frequently a low melting crystalline form was obtained which melted at 88–90°, resolidified at 125°, and then finally melted at 173–175°.

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.74; H, 10.29.

Although the possibility existed that this product could be contaminated by a methoxy derivative,[25] a negative Zeisel determination proved the absence of such derivatives.

The same product was obtained when the reaction mixture was chromatographed either on alumina or on magnesium silicate–Celite which had been dried *in vacuo* at 200° for two hours just prior to use.

When an aqueous methanolic solution of II was treated for twenty-four hours at room temperature with periodic acid, no oxidant was consumed, and starting material was recovered unchanged.

Δ^5-Cholestene-3β,4β,7α-triol Triacetate (IIIb).—The monoacetate (II) was acetylated with pyridine and acetic anhydride at room temperature overnight, during which time the product crystallized out of solution. Recrystallization from acetone yielded Δ^5-cholestene-3β,4β,7α-triol triacetate (IIIb), m. p. 172–173.5°; the mixture with II showed a depression in the m. p., 150–163°; $[\alpha]^{30}D$ −175 ± 1° (14.6 mg. in 2.00 ml. of chloroform).

Anal. Calcd. for $C_{33}H_{52}O_6$: C, 72.76; H, 9.62. Found: C, 72.83; H, 9.76.

Δ^5-Cholestene-3β,4β,7α-triol (IIIa).—Forty-five mg. of the monoacetate (II) was dissolved in 3 cc. of methanol, and to the solution was added 50 mg. of potassium carbonate dissolved in 0.5 cc. of water. After standing overnight at room temperature, the methanol was removed

(24) The melting points were taken in a Hershberg apparatus and are correct to ±1°. The carbon-hydrogen analyses were done by Dr. A. Elek, Rockefeller Institute, New York, and Dr. J. Alicino, Metuchen, New Jersey. Ultraviolet spectra were determined in 95% ethanol unless otherwise mentioned. The acid-washed alumina for chromatography was prepared according to Hollander and Gallagher, *J. Biol. Chem.*, **162**, 549 (1946).

(25) Henbest and Jones, *J. Chem. Soc.*, 1798 (1948).

in vacuo and the product extracted with ether. The ether solution was washed with water, dried over sodium sulfate, and evaporated to dryness, leaving a crystalline residue. Recrystallization twice from acetone gave the triol IIIa, m. p. 195–197°; $[\alpha]^{30}D$ −102 ± 2° (9.97 mg. in 2.00 ml. of chloroform). The analytical sample was sublimed in high vacuum.

Anal. Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.03; H, 11.11.

Acetylation of IIIa in pyridine solution at room temperature gave the triacetate, IIIb, m. p. 171–172°. An aqueous methanolic solution of 25 mg. of Δ⁵-cholestene-3β,4β,7α-triol (0.06 millimole) consumed 0.108 milliequivalent of periodic acid (corresponding to 90.5% oxidation of the glycol) in twenty-four hours at room temperature. The oxidation mixture was extracted with ethyl acetate, and the extract was washed with sodium bisulfite solution and water and dried over sodium sulfate. Evaporation of the solvent yielded 32 mg. of oil which showed an absorption maximum at 228 mμ, indicating an α,β-unsaturated carbonyl group. The semicarbazone was prepared in the usual way and melted at 195–200° with decomposition in a preheated bath. Its ultraviolet absorption spectrum showed two maxima, log $\epsilon_{232\ m\mu}$ 4.15 and log $\epsilon_{287\ m\mu}$ 3.96.[9]

Δ⁵-Cholestene-3β,4β,7α-triol Tribenzoate (IIIc).—Twenty-five mg. of the triol (IIIa) was benzoylated with 0.2 cc. of dry pyridine and 0.2 cc. of benzoyl chloride at room temperature in the usual way. The oily product was chromatographed on acid-washed alumina with ligroin, benzene:ligroin (3:1) and (1:1), and benzene. The benzene:ligroin (1:1) eluates yielded 30 mg. of Δ⁵-cholestene-3β,4β,7α-triol tribenzoate (IIIc) which on recrystallization from ether–methanol melted at 158.5–159.5°; $[\alpha]^{26}D$ −79.7° ± 4° (5.02 mg. in 2.00 ml. of chloroform).

Anal. Calcd. for $C_{48}H_{58}O_6$: C, 78.90; H, 8.20. Found: C, 78.86; H, 8.00.

3β-Acetoxy-4β-hydroxy-Δ⁵-cholesten-7-one (Va).—A solution of 200 mg. of II in 2 cc. of glacial acetic acid was oxidized with 1.4 cc. of 2% chromic acid in 85% acetic acid solution (equivalent for one hydroxyl group). After two minutes the solution turned green, and an additional 0.7 cc. of 2% chromic acid solution was added. After one hour the solution gave a positive test for chromic acid. After an additional hour, the solution was cooled in an ice-bath, and the white crystals which precipitated were collected. They were dissolved in ether, and the ether solution was washed with sodium carbonate solution, water, and dried over sodium sulfate. Upon evaporation of the ether, 84 mg. of a white solid (A) was obtained. The acetic acid filtrate was concentrated to dryness *in vacuo* and the residue dissolved in ether. The ether solution was washed with sodium carbonate solution, water, and dried over sodium sulfate. The solvent was evaporated to yield 68 mg. of gelatinous material (B).

The two fractions (A and B) were chromatographed separately on acid-washed alumina. The benzene–ether eluates from both chromatograms were combined, and after much difficulty because the compound tended to gel, a crystalline sample of 3β-acetoxy-4β-hydroxy-Δ⁵-cholesten-7-one (Va) was obtained from acetone, m. p. 197–201°; $[\alpha]^{29}D$ −77.8 ± 5° (4.11 mg. in 2.00 ml. of chloroform) log $\epsilon_{235\ m\mu}$ 4.04. The infrared spectral analysis showed the presence of hydroxyl, acetoxyl, and α,β-unsaturated carbonyl groups.

In another run, exactly 4 equivalents of chromic acid reagent (sufficient to oxidize two hydroxyl groups) were added and the reaction mixture allowed to stand at room temperature for twenty-four hours. Excess oxidant was still present at the end of this period, and the sole product isolated was the monoketone (Va) identical with that obtained above.

3β,4β-Diacetoxy-Δ⁵-cholesten-7-one (Vb).—To a solution of 84 mg. of monoacetate, Va, in 0.5 cc. of pyridine was added 0.5 cc. of acetic anhydride, and the solution was allowed to stand overnight at room temperature. The mixture was poured onto ice and then extracted with ether. The ether solution was washed with dilute sulfuric acid, sodium carbonate solution, water, and dried over sodium sulfate. Evaporation of the solvent gave 104 mg. of a white product. Again recrystallization was made difficult due to the jelly-like character of the compound. A crystalline sample of 3β,4β-diacetoxy-Δ⁵-cholesten-7-one (Vb) was obtained from ether–methanol and melted at 215–225°; $[\alpha]^{29}D$ −128 ± 9° (2.17 mg. in 2.00 ml. of chloroform) log $\epsilon_{231\ m\mu}$ 3.97. The diacetate ketone was sublimed at high vacuum for analysis.

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 74.00; H, 9.42. Found: C, 74.36; H, 9.67.

Δ⁴,⁶-Cholestadien-3-one (6-Dehydrocholesten-3-one) (IV).—A mixture of 241 mg. of 3β-acetoxy-Δ⁵-cholestene-4β,7α-diol (II), 0.6 cc. of cyclohexanone and 65 cc. of toluene was dried by distilling the toluene until the distillate was clear. To the dry solution was added 1.4 g. of aluminum *t*-butylate and the mixture refluxed for five hours. Ice was then added and the mixture steam distilled. The distilland was extracted twice with ether, and the ether solution was washed with 5% sulfuric acid solution, 5% sodium carbonate solution, water and dried over sodium sulfate. The oily residue (252 mg.) remaining after evaporation of the solvent was fractionated using Girard reagent T to give 46 mg. of non-ketonic and 193 mg. of ketonic materials. The ketonic fraction was chromatographed on acid-washed alumina. The benzene–ligroin (1:9, 1:3, and 1:1) eluates yielded 27, 46, and 24 mg., respectively, of yellow oil, all of which showed absorption maxima at 285 mμ and gave red 2,4-dinitrophenylhydrazones.

The benzene–ligroin (1:3) fraction (46 mg.) was sublimed in high vacuum at 120–145° to give 31 mg. of oil which crystallized from ether–methanol, yielding 6-dehydrocholesten-3-one, m. p. 80–80.5°; log $\epsilon_{285\ m\mu}$ 4.41. The mixed m. p. with an authentic sample was not depressed, and infrared spectral analysis confirmed its identity.

The **semicarbazone** was prepared in the usual way, and after recrystallization from ethanol 6-dehydrocholesten-3-one semicarbazone melted at 235–240° with decomposition; the m. p. determined on a Kofler block was 210–218° dec.; log $\epsilon_{300\ m\mu}$ 4.64.

Anal. Calcd. for $C_{28}H_{45}N_3O$: C, 76.50; H, 10.31. Found: C, 76.75; H, 10.66.

The **2,4-dinitrophenylhydrazone** was prepared in the usual way and after crystallization from chloroform–methanol melted at 224–226° (from ethyl acetate it melted at 230–233°); log $\epsilon_{398\ m\mu}$ 4.57 (chloroform).

Anal. Calcd. for $C_{33}H_{46}O_4N_4$: C, 70.43; H, 8.23. Calcd. for $C_{33}H_{46}O_4N_4 \cdot CH_3OH$: C, 68.66; H, 8.48. Found: C, 69.05; H, 8.14.

3β,4β-Dihydroxy-Δ⁵-cholesten-7-one (Vc) and its Dibenzoate (Vd).—One tenth g. of II was dissolved in 2 cc. of *t*-butanol containing 0.1 cc. of water and 0.3 cc. of pyridine. The solution was treated with 120 mg. of N-bromoacetamide and the reaction mixture allowed to stand eighteen hours at room temperature. After destroying the excess N-bromoacetamide, the mixture was extracted with ether. The organic extract was washed with sulfuric acid, sodium carbonate solution, water, and dried over sodium sulfate. Evaporation of the solvent left 103 mg. of crystalline residue which exhibited an absorption maximum at 234 mμ. Infrared spectral analysis indicated that this product was identical with the monoketone (Va) obtained from the chromic acid oxidation.

The residue was saponified by dissolving it in 20 cc. of methanol containing 200 mg. of potassium carbonate and 2 cc. of water. After standing at room temperature overnight, the product was precipitated as a curd by the addition of water and was extracted into ether. The ether extract was washed neutral, and on evaporation of the solvent, a gelatinous material remained. By leaching several times with boiling ligroin (b. p. 60°) an insoluble powder (16 mg.) was obtained which melted at 199–205°; $[\alpha]^{25}D$ −60.6° ± 2° (8.58 mg. dissolved in 2.00 ml. of

chloroform). It exhibited an absorption maximum at 234.5 mμ. Petrow and Starling reported[7] a m. p. of 205–206°; $[\alpha]_D$ −71.4°.

This sample of 3β,4β-dihydroxy-Δ^5-cholestan-7-one (Vc) was benzoylated in pyridine solution at room temperature. The oily product was purified by chromatography on alumina; a benzene–ligroin (1:1) solution eluted 13 mg. of crystalline material. Two recrystallizations from ethanol gave the dibenzoate, Vd, as prisms, m. p. 144–144.5°; $[\alpha]^{26}D$ −47.4° ± 4° (5.06 mg. in 2.00 ml. of chloroform); log $\epsilon_{232\,m\mu}$ 4.57.

Treatment of 3β-Acetoxy-Δ^5-cholesten-4β-ol (VIa) with N-Bromoacetamide.—To 100 mg. of 3β-acetoxy-Δ^5-cholesten-4β-ol (VIa)[20] in 3 cc. of pyridine and 0.2 cc. of water was added 53 mg. of N-bromoacetamide (twice the theoretical amount). The solution was allowed to stand at room temperature for twenty-one hours, and then the mixture was extracted with ether. The ether solution was washed with 5% sulfuric acid solution, 5% sodium carbonate solution, and with water. After drying over anhydrous sodium sulfate, the solvent was evaporated leaving 110 mg. of crystalline residue. This material (m. p. 180–190°) appeared to consist mainly of the starting compound, but in order to detect even small amounts of the oxidized product, 3β-acetoxy-Δ^5-cholesten-4-one (m. p. 124°),[21] it was dissolved in benzene:ligroin (1:3) and chromatographed on acid-washed alumina. Elutions with benzene:ligroin (1:3), benzene:ligroin (1:1), benzene, ether:benzene (1:1), and methanol:ether (1:19) gave 5 mg. (oil), 49 mg. (crystals), 3 mg. (crystals), 28 mg. (crystals) and 9 mg. (oil), respectively. The second and third fractions were combined and recrystallized from acetone to give 3β-acetoxy-Δ^5-cholesten-4β-ol, m. p. 190–193°. The fourth fraction was recrystallized from ligroin (b. p. 30°) and melted at 161–163°. This product exhibited no absorption in the ultraviolet between 220–320 mμ and was proved to be 4β-acetoxy-Δ^5-cholesten-3β-ol (VIc)[20] by mixed melting point and by infrared spectral analysis. Acetylation gave the 3β,4β-diacetate (VId),[26] m. p. 167–168°, which did not depress the melting point of the diacetate prepared from the 3-monoacetate. The 3-monoacetate of the *cis* diol readily rearranges to the 4-monoacetate, and, as described below, this migration on alumina represents a convenient preparation of the latter compound.

In another run, 100 mg. of the 3β-acetoxy-Δ^5-cholesten-4β-ol was treated for eighteen hours at room temperature with 66 mg. of N-bromoacetamide in 5 cc. of *t*-butanol containing 0.5 cc. of pyridine and 0.1 cc. of water. The product was again chromatographed, and in this way 45% of the material was recovered as the 3β-acetoxy derivative and 40% as the 4β-acetoxy compound. The chromatographic eluates were all examined in the ultraviolet region between 220–320 mμ, and none exhibited any absorption.

In a third experiment the oxidation was carried out in pyridine solution for sixty hours at room temperature. The residue obtained from the neutral ether extract was crystalline and weighed 61 mg. After recrystallization from methanol, it melted at 190–191° and did not depress the melting point of the 3β-acetoxy derivative.

The Chromic Acid Oxidation of 3β-Acetoxy-Δ^5-cholesten-4β-ol.—To a suspension of 50 mg. of 3β-acetoxy-Δ^5-cholesten-4β-ol (VIa) in 1 cc. of glacial acetic acid, a solution of 0.5 cc. of 2% chromic acid in acetic acid was added. In five minutes the chromic acid was all consumed, and an additional 0.5 cc. was added. After fifty-five minutes titration with 0.0970 *N* thiosulfate showed that 1.92 atoms of O was consumed. The titration mixture was extracted with ether and the ether solution washed with sodium carbonate solution, water, and dried. Evaporation of the solvent gave 50 mg. of crystalline product which upon recrystallization from ether–methanol gave 5,6-epoxy-3β-acetoxycholestan-4-one, m. p. 165–167° (Kofler block); reported[21] 173–174°.

Treatment of 3β-Benzoxy-Δ^5-cholestan-4β-ol (VIb) with N-Bromoacetamide.—To 100 mg. of 3β-benzoxy-Δ^5-cholesten-4β-ol (VIb)[26] in 4.3 cc. of pyridine and 3 drops of water was added 58 mg. of N-bromoacetamide and the mixture allowed to stand for eighteen hours at room temperature. The reaction mixture was worked up as usual to give a crystalline product which was dissolved in benzene:ligroin (1:3) and chromatographed on acid-washed alumina. Elutions with benzene–ligroin (1:3), benzene–ligroin (1:1), benzene, ether, and methanol–ether (1:49) gave 53, 30, 6, 13, and 6 mg. of material, respectively. Recrystallization of the first eluate from acetone resulted in plates melting at 210–211°, and recrystallization of the second eluate gave crystals, m. p. 205–208°. Neither gave depression when mixed with the starting material, 3β-benzoxy-Δ^5-cholesten-4β-ol (m. p. 209–212°). The third and fourth fractions were crystalline but melted unsharply between 160–190°. They exhibited no absorption in the ultraviolet region for an α,β-unsaturated carbonyl group and gave negative tests with Brady reagent (dinitrophenylhydrazine).

4β-Acetoxy-Δ^5-cholesten-3β-ol (VIc). Preparation.—4β-Acetoxy-Δ^5-cholesten-3β-ol, m. p. 163–165°, was prepared from cholesterol acetate and selenium dioxide in acetic acid solution.[20] This compound may also be prepared conveniently from the 3-monoacetate (VIa) by acyl migration on alumina, a rearrangement which has already been observed to occur in hot acetic acid.[20] A solution of 200 mg. of VIa in 20 cc. of benzene:ligroin (1:3) was poured through a column of 6 g. of acid-washed alumina. After one and one-half hours, the column was developed, and three fractions were obtained. The first, eluted with 120 cc. of benzene:ligroin (1:3) and 200 cc. of benzene:ligroin (1:1), consisted of 70 mg. of unchanged 3-monoacetate, m. p. 185–186°. The second fraction, eluted with 80 cc. of benzene, weighed 19 mg. and was a mixture of the 3- and the 4-monoacetates. The third fraction, eluted with 40 cc. of benzene, 40 cc. of ether:benzene (1:1), 150 cc. of ether, and 50 cc. of methanol:ether (1:19), consisted of 119 mg. of the 4-monoacetate, m. p. 164.5–166°.

In a run in which 2.00 g. of VIa was rearranged on 60 g. of alumina, 1.21 g. of the pure 4-monoacetate and 805 mg. of a mixture of the two isomers were obtained.

Treatment with N-Bromoacetamide.—To 200 mg. of 4β-acetoxy-Δ^5-cholesten-3β-ol in 5 cc. of pyridine and 0.4 cc. of water was added 126 mg. of N-bromoacetamide and the mixture allowed to stand for twenty-one hours at room temperature. The oxidation mixture was worked up as previously described, and the 205 mg. of crystalline product was chromatographed on acid-washed alumina. From the benzene and ether–benzene eluates was obtained 178 mg. (89%) of crystalline 4β-acetoxy-Δ^5-cholesten-3β-ol, which upon recrystallization from ligroin melted at 163–164° and did not depress the melting point of the starting material. Four mg. of oil was eluted with ligroin, and 16 mg. of yellow oil was found in the ether and methanol:ether eluates, but these could not be crystallized.

5,6-Epoxy-3β-acetoxycholestan-4β-ol (VII). Preparation.—To a solution of 150 mg. of 3β-acetoxy-Δ^5-cholesten-4β-ol (VIa) in 3 cc. of benzene and 1 cc. of chloroform was added 3 cc. of a benzene solution containing 151 mg. of perbenzoic acid. The reaction mixture was allowed to stand in the refrigerator for three days, during which time 46.8 mg. of perbenzoic acid was consumed (theory 46.6 mg.). The oxidation mixture was taken up in ether and the ether solution washed with sodium bicarbonate solution and water. The extract was dried over sodium sulfate and the ether evaporated to give 163 mg. of crystalline product. Recrystallization from ether:methanol and ether:acetone yielded analytically pure 5,6-epoxy-3β-acetoxycholestan-4β-ol (VII), m. p. 194–196°; $[\alpha]^{32}D$ −30.9 ± 1° (8.42 mg. in 2.00 ml. of chloroform). There was no depression of melting point when mixed with the starting material (VIa).

Anal. Calcd. for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.64; H, 10.49.

Acetylation of the epoxy monoacetate (VII) with acetic anhydride in pyridine overnight at room temperature gave

(26) Rosenheim and Starling, *J. Chem. Soc.*, 377 (1937).

the diacetate, m. p. 174–175°; $[\alpha]^{31}D$ −22.1 ± 3° (3.18 mg. in 2.00 ml. of chloroform); reported[26] m. p. 178–179°; $[\alpha]_D$ −22°.

Oxidation with Chromic Acid.—Thirty mg. of 5,6-epoxy-3β-acetoxycholestan-4β-ol (VII), m. p. 189–192°, was oxidized with chromic acid in acetic acid solution. The product obtained was the 4-keto epoxide, m. p. 165–167° (Kofler block), identical with that obtained by chromic acid oxidation of the 3β-acetoxy-Δ⁵-cholesten-4β-ol (VIa), m. p. 165–167° (Kofler block).

Treatment with N-Bromoacetamide.—To a solution of 100 mg. of 5,6-epoxy-3β-acetoxycholestan-4β-ol (VII) in 2 cc. of pyridine and 0.1 cc. of water was added 100 mg. of N-bromoacetamide. The mixture was allowed to stand at room temperature for twenty-one hours. It was extracted in the usual way with ether, yielding 100 mg. of crystalline residue. Recrystallization from methanol yielded 50 mg. of the starting material, m. p. 194–195°; mixed m. p. showed no depression. The material in the mother liquor was chromatographed on alumina, and the uniform product weighing 49 mg. was eluted with ligroin: benzene (1:1) and benzene. Recrystallization from methanol gave VII, m. p. 190–191°.

3β-Acetoxycholestan-4β-ol (VIII). Preparation.—Two hundred seven mg. of cholestane-3β,4β-diol[26] was dissolved in 2.5 cc. of benzene containing 0.3 cc. of pyridine and treated with 0.06 cc. of acetic anhydride (theoretical for 1 hydroxyl). After eighteen hours the reagents were removed *in vacuo* and the residue taken up in ether. The ether extract was washed neutral with sulfuric acid solution, sodium carbonate solution, water, and dried over sodium sulfate. Evaporation of the solvent left a crystalline residue weighing 219 mg. The residue was extracted five times with boiling ligroin (b. p. 30°) in order to separate the insoluble diol (75 mg.) from the acetate. The ligroin-soluble material was recrystallized three times from acetone, giving an analytical sample melting at 200–205°; $[\alpha]^{24}D$ +13.1 ± 2° (5.34 mg. in 2.00 ml. of chloroform).

Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found: C, 78.18; H, 11.20.

The material in the acetone mother liquor was combined with similar fractions from other runs and was purified by chromatography on alumina. Additional amounts of the monoacetate were obtained in the benzene:ligroin (1:9) eluates, but they were contaminated with small amounts of the diacetate (m. p. 134–135°).[26] Recrystallization from acetone afforded satisfactory samples, m. p. 195–197°.

Oxidation with Chromic Acid.—To a suspension of 50 mg. of 3β-acetoxycholestan-4β,ol (VIII) in 1 cc. of glacial acetic acid, 0.8 cc. of 2% chromic acid–acetic acid solution was added. After two and one-half hours the solution was poured onto ice and water and extracted with ether. The ether solution was washed repeatedly with water and dried. Evaporation of the ether gave 48 mg. of crystalline product which upon recrystallization from acetone gave 3β-acetoxycholestan-4-one, m. p. 114–116°. The analytical sample was recrystallized from ether–acetone, m. p. 117–118.5°; $[\alpha]^{32}D$ −22.9 ± 4° (2.63 mg. in 2.00 ml. of chloroform).

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 78.62; H, 10.62.

Treatment with N-Bromoacetamide.—To 112 mg. of 3β-acetoxycholestan-4β-ol (VIII) in 3 cc. of pyridine and 0.2 cc. of water was added 100 mg. of N-bromoacetamide and the solution allowed to stand for twenty-one hours at room temperature. The reaction mixture was worked up in the usual way, yielding 108 mg. of product which was purified by chromatographic analysis. From the ligroin and benzene–ligroin (1:3) eluates, 85 mg. of low melting crystals, m. p. 91–104°, was obtained. The benzene–ligroin (1:1), benzene, and ether eluates yielded 21 mg. (18.5%) of crystals, m. p. 195–200°, which did not depress the melting point of the starting material, 3β-acetoxycholestan-4β-ol (VIII). The low melting fraction was rechromatographed on alumina, and 49 mg. (44%) of 3β-acetoxycholestan-4-one was eluted with ligroin and benzene–ligroin (1:19 and 1:9). Recrystallization from acetone gave 3β-acetoxycholestan-4-one, m. p. 114–116°, which did not depress the m. p. of the 4-keto compound (117–118.5°) obtained by the chromic acid oxidation of 3β-acetoxycholestan-4-ol (VIII). The later fractions (benzene–ligroin (1:9) and the benzene eluates) consisted of 35 mg. of a mixture of the 4-ketone and the starting material.

The oxidation was repeated on 47.8 mg. of 3β-acetoxycholestan-4β-ol (VIII) in 1.5 cc. of pyridine and 0.1 cc. of water using 47.8 mg. of N-bromoacetamide for twenty-four hours at room temperature. At this time, an additional 47.8 mg. of the oxidant was added, and then the mixture was allowed to stand for another forty hours. The product was chromatographed on alumina in the usual way to afford 73% of the oxidized product, 3β-acetoxycholestan-4-one and 25% of a mixture of the 4-ketone and the starting material.

Summary

The evidence for assigning the structure, 3β-acetoxy-Δ⁵-cholestene-4β,7α-diol, to the product obtained from the reaction of cholesterol acetate with excess N-bromosuccinimide is presented. During this investigation the observation has been made that some secondary hydroxyl groups which have vicinal substituents are resistant to oxidation. The mechanism of this inhibition to oxidation is discussed.

NEW YORK 21, N. Y. RECEIVED MAY 1, 1950