is heated with hydroxylamine hydrochloride (4.1 g.) and anhydrous sodium acetate (4.1 g.) in 200 cc. of absolute ethanol under reflux for four hours. After removal of the sodium chloride the ethanol is evaporated in vacuum. The residue is taken up in 200 cc. of absolute ether. On passing dry hydrogen chloride through the solution the oxime hydrochloride is deposited in form of a sticky oil, which completely crystallizes after twenty hours in the ice box, m. p. 99°.

2-Phenyl-3-aminobutane.—The oxime hydrochloride (4.7 g., 0.2 mole) of 50 cc. of glacial acetic acid with 0.5 g. of platinum oxide takes up somewhat less than three moles of hydrogen. The solvent is removed in vacuum. The residue is taken up in ether, the base extracted with dilute hydrochloric acid, liberated with alkali, and taken up in ether. The amine was obtained as an oil (1.8 g., 60%).

3,4-Dimethyldihydroisoquinoline.—The amine (1.8 g.) is heated with 20 cc. of 87% formic acid on the steam-bath for two hours. The formic acid is evaporated in vacuum and the procedure repeated. The resulting crude formylamino compound still contains some amine which is extracted with dilute acetic acid. The ethereal solution is evaporated to dryness. The carefully dried crude formylamino compound (1.1 g.) is dissolved in 30 cc. of freshly distilled tetralin and treated with 3.5 g. of phosphorus pentoxide. The mixture is refluxed for thirty minutes and another 3.5 g. of pentoxide is added in the middle of this time. The resulting base is isolated in the usual manner and distilled at 120° (10 mm.). It is converted into the picrate and recrystallized from acetone as needles, m. p. 208°.

Anal. Calcd. for $C_{11}H_{13}N \cdot C_6H_3O_7N_3$: C, 52.57; H, 4.12. Found: C, 52.80; H, 4.12.

The hydrochloride is prepared from the picrate by trituration with dilute alkali and extraction with ether. When hydrogen chloride is passed through the dry ethereal solution the hydrochloride crystallizes and forms beautiful needles from ethanol, m. p. 208° (sublimes).

3,4-Dimethylisoquinoline Picrate.—As in the case of the 1,3-dimethyl compound palladium (220°, thirty minutes) easily dehydrogenates the dihydro base in almost quantitative yield. The picrate crystallized immediately from the aqueous solution in short needles, m. p. 224–226°.

Anal. Calcd. for $C_{11}H_{11}N \cdot C_6H_3O_7N_3 \cdot H_2O$: C, 50.37; H, 3.95. Found: C, 50.37; H, 3.69.

Acknowledgment.—The author is indebted for support of this work to Prof. L. F. Fieser in whose laboratory part of this work was performed.

Summary

Gelsemine can be degraded to skatole and a base $C_{11}H_{11}N$ which is considered to be a dimethylisoquinoline. None of the three possible dimethylisoquinolines bearing the methyl groups in the pyridine part of the molecule is identical with the base from gelsemine.

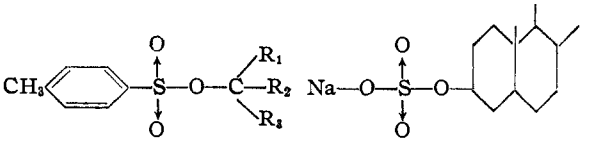
CONVERSE MEMORIAL LABORATORY
CAMBRIDGE 38, MASS. RECEIVED SEPTEMBER 2, 1947

[CONTRIBUTION FROM THE DIVISION OF HORMONE CHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Studies in Steroid Metabolism. V. The Problem of Walden Inversion in the Reactions of Steroid Hydrogen Sulfates and Steroid Sulfites[1,2]

BY SEYMOUR LIEBERMAN, LUCIE B. HARITON AND DAVID K. FUKUSHIMA

This paper deals with the problem of Walden inversion in the reactions of steroid hydrogen sulfates and with the point of cleavage of the S-O-R linkage in these compounds. Since urinary steroids are excreted, at least in part, as water-soluble sulfates,[3] this problem is of biological interest as well as chemical. The isolation of these conjugates is difficult, their quantitative estimation impractical, and therefore it is the general practice to hydrolyze these conjugates with boiling acid in order to estimate or identify the free steroids. Although it has been recognized that various artefacts result from this hydrolytic procedure, the possibility that another type of transformation product might be formed has been overlooked. This type of artefact would result from a Walden inversion accompanying the hydrolysis of those urinary steroids conjugated with sulfuric acid. The supposition is based on the results obtained on the cleavage of analogous sulfonoxy compounds.



I II

Kenyon and Phillips[4] have shown that displacement reactions of *p*-toluenesulfonates of optically active alcohols (I) are accompanied by inversion of configuration, and there are at least three reports[5] demonstrating that steroid toluenesulfonates undergo displacement reactions accompanied by Walden inversion. Esters of sulfonic acids, therefore, unlike esters of carboxylic acids react by a rupture of the alkyl oxygen (SO-R) linkage.

(1) This paper was presented before the Division of Organic Chemistry at the 112th Meeting of the American Chemical Society, New York City, September, 1947.

(2) A portion of this paper was taken from the Master's Thesis of Lucie B. Hariton, June, 1947, Department of Chemistry, New York University.

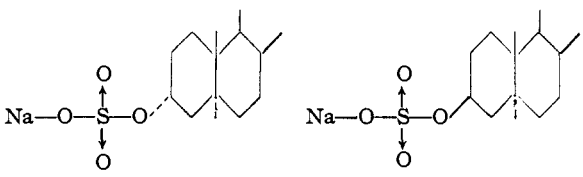
(3) The following steroids have been isolated from urine as their sulfuric acid esters: (a) Estrone [Schachter and Marrian, *J. Biol. Chem.*, **126**, 663 (1938)]; (b) androsterone [Venning, Hoffman and Browne, *J. Biol. Chem.*, **146**, 369 (1942)]; (c) dehydroisoandrosterone [Munson, Gallagher and Koch, *ibid.*, **152**, 67 (1944)]; (d) Δ^{16}-allopregnenol-3β-one-20 [Klyne and Marrian, *Biochem. J.*, **39**, Proc. xiv (1945)]; (e) uranediol [Klyne, *ibid.*, **40**, Proc. lv (1946)].

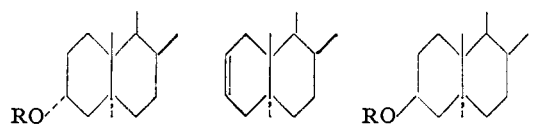
(4) Kenyon, Phillips, *et al.*, *J. Chem. Soc.*, **123**, 44 (1923); **127**, 399, 2552 (1925); 1676 (1930); 1072, 1663 (1935); *Trans. Faraday Soc.*, **26**, 451 (1930).

(5) (a) Prelog and Szpilfogel, *Helv. Chim. Acta*, **27**, 390 (1944); (b) Plattner and Furst, *ibid.*, **26**, 2226 (1943); (c) Gallagher, private communication.

It has been generally assumed[6] that esters of sulfuric acid likewise are cleaved at the SO-R linkage. The 6-sulfates of various hexoses when treated with barium hydroxide for long periods at 100° gave 3,6-anhydro sugars,[7] a result which may be interpreted to indicate that the SO-R linkage had been ruptured. If the urinary steroid sulfates (II) are similarly cleaved at the SO-R linkage by acid hydrolysis, it would be expected that Walden inversion would occur and as a consequence, a steroid which had been excreted as the 3β-sulfate would be isolated and identified after acid hydrolysis as a 3α-hydroxy compound. The direction of cleavage of these steroid sulfates is therefore of considerable biological importance and in addition affords information on the general problem of cleavage of esters.

We have studied the fission of the S-O-R linkage by examining the products obtained from the acid hydrolysis and displacement reactions of sulfuric acid esters of several steroid alcohols. The acid hydrolysis has been accomplished by continuous ether extraction of a suspension of the steroid sulfate in aqueous acid. Under these conditions the free steroid accumulates in the ether extract and it is therefore unnecessary to resort to the prolonged heating usually employed for the acid hydrolysis of urinary steroid conjugates. The ether soluble reaction products were separated and were identified by melting point, melting point of mixtures and by their characteristic infrared spectra. Mother liquors and all non-crystalline residues were also submitted to infrared spectral analysis in order that every precaution be made to detect small amounts of an isomer resulting from a reaction involving Walden inversion. Under these conditions, cholestanol-3α sulfate (III) and cholestanol-3β sulfate (IV) yielded the corresponding alcohols (Va and VIIa) without any inversion of configuration. Hydrolysis of the sulfates of cholesterol and dehydroisoandrosterone was similarly accomplished without inversion of configuration.



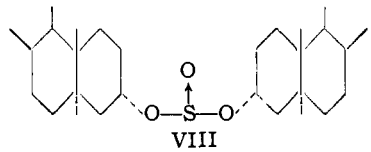


Va, R = H
Vb, R = CH_3CO

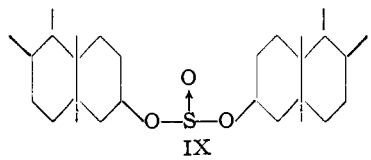
VI

VIIa, R = H
VIIb, R = CH_3CO

In view of these results, a series of reactions were investigated under conditions favorable for a displacement reaction with inversion of configuration. Sodium cholestanol-3α sulfate (III) was heated with silver acetate in acetic acid, and from the reaction mixture, cholestanol-3α (Va) and its acetate (Vb), together with a small amount of neocholestene (VI) were obtained as the reaction products. The isomeric sodium cholestanol-3β sulfate (IV) when treated with silver acetate in acetic acid also yielded products (VIIa and VIIb) without inversion. The results indicated that these reactions were also accomplished with retention of configuration about C_3. Sodium cholestanol-3α sulfate (III) and sodium cholestanol-3β sulfate (IV) when treated at room temperature with dry hydrogen chloride in absolute methanol solution, were converted in high yield to the corresponding alcohols (Va and VIIa) without any evidence of inversion about the asymmetric carbon atom.



VIII



IX

In order to determine whether cleavage of the S-OR linkage was a general phenomenon, we have investigated the cleavage of alkyl sulfites. The sulfites of cholestanol-3α (VIII) and cholestanol-3β (IX) were treated with silver acetate in acetic acid and with dry hydrogen chloride in methanol precisely as were the sulfates, and the products were isolated as in the previous experiments. Here, also, the products retained their configuration about the asymmetric carbon atom and no evidence of Walden inversion was observed.

Discussion

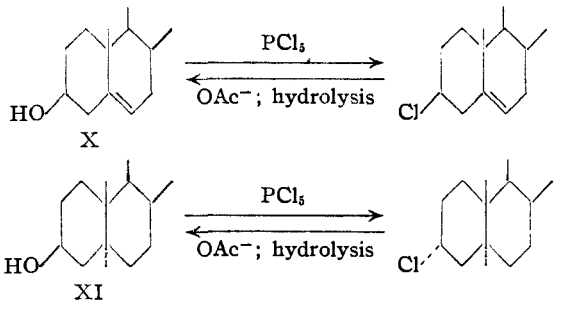
The complete retention of configuration in all of the foregoing replacement reactions of the sulfates and the sulfites indicates that the principal course of the reaction was cleavage of the S–OR bond. Double inversion cannot account for retention of configuration because both the sulfates and sulfites were prepared by methods which do not involve the O–R bond. The reagents appear always to have attacked the sulfur rather than the asymmetric carbon atom. In the acid-catalyzed reaction, it may be postulated that a proton adds to the oxygen between the sulfur and carbon atoms. This positive charge weakens the S–OR bond since there already exists a positive formal charge of two on the sulfur; the sulfur–oxygen linkage is then cleaved by an attack of a solvent molecule (water or methanol) on the sulfur atom yielding the alcohol with the original configuration.

(6) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 355.

(7) Percival, *J. Chem. Soc.*, 119 (1945).

That a carbonium ion was formed as an intermediate is possible, but unlikely, from the experimental results. The possibility exists that a carbonium ion is so oriented by virtue of the many asymmetric centers in the steroid nucleus that the entering group could attack the carbonium carbon from one side preferentially. If this were the case, both the isomeric α- and β-sulfates would yield a common carbonium ion and would consequently yield the same isomer or mixture of isomeric alcohols. Since each sulfate was hydrolyzed with complete retention of configuration, this mechanism is unlikely and this view is strengthened by the results of the experiments in methanol with dry hydrogen chloride. Had these reactions taken place by a carbonium ion mechanism, the products would have been the methyl ethers which were in no case isolated. These results eliminate not only the carbonium ion as a possible intermediate but also exclude the displacement reaction involving a cleavage of the SO-R linkage.

In view of some recent work of Shoppee, who has pointed out that derivatives of the unsaturated series[8] (X) react differently from those of the saturated series[9] (XI), the hydrolysis of the sulfates of the two unsaturated steroids, cholesterol and dehydroisoandrosterone cannot be unobjectionably interpreted with respect to the direction of cleavage of the S–O–R linkage.



Replacement of chlorine by acetoxyl and replacement of hydroxyl by chlorine in derivatives of the unsaturated series (X) was accomplished with retention of configuration, whereas these replacement reactions were accompanied by inversion with the saturated compounds (XI) cholestane and androstane. Since the presence of the β,γ-double bond influences the steric course of reactions at the asymmetric C_3, most of the reactions reported here were carried out with derivatives of the saturated steroids in order to facilitate the interpretation of the experimental results.

It is important to note that in the reactions with silver acetate in acetic acid the uninverted **alcohol** was isolated. The cholestanol acetates found among the reaction products were probably not produced by the displacement of the sulfate ion by the acetate ion. Such a displacement must lead to inversion, and since this was not observed in any experiment, the ester must result from the secondary acetylation of the alcohol produced by fission of the sulfate. In agreement with this interpretation we have found that cholestanol-3α acetate is formed from cholestanol-3α under the same experimental conditions employed for the cleavage, whereas cholestanol-3α acetate was recovered unchanged when subjected to this treatment. The formation of the uninverted **alcohols** from the reactions with silver acetate can only be explained by the direct cleavage of the S–OR linkage. In addition to this, it is apparent that under the somewhat more drastic conditions utilized in these reactions, some cleavage of the SO-R bond has occurred as evidenced by the formation of small amounts of neocholestene.

While the formation of unsaturated substances is without significance for the problem of Walden inversion in the displacement reaction, it has considerable interest because similar unsaturated derivatives of steroids have been frequently isolated from urine. Among these are the compounds: $\Delta^{2(\text{or }3)}$-androstenone-17,[10] $\Delta^{3,5}$-androstadienone-17,[11] Δ^{9}-etiocholenol-3α-one-17[12] and Δ^{9}-androstenol-3α-one-17.[13] The first two of these substances very probably arise from androsterone and dehydroisoandrosterone which have been shown to be, in part at least, excreted as sulfates in human urine. In our experiments, neocholestene was found in only those reactions conducted at elevated temperature and it is likely that it is produced from the steroid sulfate by an elimination reaction. The unsaturated steroid derivatives which are isolated from urine after hydrolysis at reflux temperature are also probably formed by such an elimination reaction and need not necessarily be produced by the prolonged action of the hot acid on the free steroid alcohols resulting from the cleavage of the conjugates. This contention is supported by the results of Talbot, Ryan and Wolfe[14] who, working with the sulfate of dehydroisoandrosterone reported that "acid hydrolysis damages the conjugated dehydroisoandrosterone sterone before hydrolysis to the unconjugated form." Since it appears that temperature is the factor which favors the elimination reaction of these steroid sulfates, the hydrolytic procedure employed in this investigation involving only a room temperature extraction offers marked advantage for the circumvention of this undesirable reaction.

Acknowledgment.—This work has been supported by the Jane Coffin Childs Memorial Fund

(8) Shoppee, *J. Chem. Soc.*, 1147 (1946).

(9) Shoppee, *ibid.*, 1138 (1946).

(10) Hirschmann, *J. Biol. Chem.*, **136**, 483 (1940).

(11) Burrows, Cook, Roe and Warren, *Biochem. J.*, **31**, 950 (1937).

(12) Dobriner, Lieberman, Hariton, Sarett and Rhoads, *J. Biol. Chem.*, **169**, 221 (1947).

(13) (a) Lieberman, Dobriner and Rhoads, *Federation Proc.*, **6**, 270 (1947); (b) Dorfman, Shiller and Sevringhaus, *Endocrinology*, **37**, 262 (1945); Miller, Dorfman and Sevringhaus, *ibid.*, **38**, 19 (1946).

(14) Talbot, Ryan adnd Wolfe, *J. Biol. Chem.*, **148**, 593 (1943).

for Medical Research, the Commonwealth Fund, the New York Foundation Fund, the Lillia Babbit Hyde Foundation and the Albert and Mary Lasker Foundation, Inc. We would also like to express our appreciation to Dr. T. F. Gallagher for his generous assistance in the preparation of the manuscript.

Experimental[15,16]

Sodium Cholesterol Sulfate.—This compound was prepared with pyridine sulfur trioxide according to the method of Sobel[17] and crystallized from methanol–ether as shiny plates, m. p. 182–183°. It was dried for analysis at 100° *in vacuo* for twenty-four hours.

Anal. Calcd. for $C_{27}H_{45}O_4SNa \cdot 3H_2O$: Na, 4.23. Found: Na, 4.14.

Potassium Dehydroisoandrosterone Sulfate.—This substance was prepared according to the method of Sobel.[17] After several recrystallizations from ethanol–ether, potassium dehydroisoandrosterone sulfate melted at 221–223°.

Anal. Calcd. for $C_{19}H_{27}O_5SK$: K, 9.6; S, 7.88. Calcd. for $C_{19}H_{27}O_5SK \cdot H_2O$: K, 9.2; S, 7.55. Found: K, 9.23; S, 7.70.

Sodium Cholestanol-3β Sulfate.—To a cold solution of 1 g. of cholestanol-3β in 30 cc. of dry ether, 0.4 cc. of chlorosulfonic acid was added dropwise with swirling. After the mixture stood for one hour at room temperature, the ether was removed by distillation under reduced pressure. To the residue 4 cc. of 2 *N* sodium hydroxide solution was added slowly while the mixture was cooled in an ice-bath. The crystalline precipitate which formed was collected on a Büchner funnel, washed several times with ether and dried (1.15 g., 91%). Several recrystallizations from methanol yielded pure sodium cholestanol-3β sulfate, m. p. 174–175.5°; $[\alpha]^{29}D$ +16.6 ± 4° (ethanol).

Anal. Calcd. for $C_{27}H_{47}O_4SNa$: C, 66.08; H, 9.65; S, 6.53; Na, 4.69. Found: C, 66.04; H, 9.72; S, 6.61; Na, 4.52.

Sodium Cholestanol-3α Sulfate.—This sulfate was prepared in 76% yield according to the method of Sobel.[17] After three recrystallizations from methanol, sodium cholestanol-3α sulfate melted at 136–137°; $[\alpha]^{29}D$ +15.0 ± 5° (ethanol).

Anal. Calcd. for $C_{27}H_{47}O_4SNa$: C, 66.08; H, 9.65; S, 6.53; Na, 4.69. Found: C, 65.91; H, 9.54; S, 6.39; Na, 4.55.

It was also prepared in 95% yield using chlorosulfonic acid as described for cholestanol-3β sulfate. In general the preparation of the steroid sulfates by the chlorosulfonic acid method was more convenient and gave higher yields than the pyridine sulfur trioxide method.

Cholestanol-3β Sulfite.—To a solution of 1.5 g. of cholestanol-3β in 25 cc. of dry ether and 1 cc. of pyridine was added slowly 5 cc. of dry ether containing 0.2 cc. of thionyl chloride. After standing overnight at room temperature, an equal volume of ether was added to the mixture. The ether solution was washed with dilute hydrochloric acid solution, water, sodium bicarbonate solution and water and dried over sodium sulfate. The ether was then evaporated and the residue digested with 50 cc. of ethanol. The hot suspension was filtered to separate the insoluble starting material (650 mg.), m. p. 135–139°. The filtrate was evaporated to dryness and the residue (720 mg.) was digested in 100 cc. of hot acetone. On cooling, 690 mg. of platelets, m. p. 196–197°, was obtained. Cholestanol-3β sulfite was recrystallized for analysis from ethyl acetate and dried overnight in vacuo at 100°; m. p. 196–197.5°; reported[9] 194°; $[\alpha]^{31}D$ +5.2 ± 2.5° (chloroform).

Anal. Calcd. for $C_{54}H_{94}O_3S$: C, 78.77; H, 11.51; S, 3.89. Found: C, 78.71; H, 10.91; S, 3.97.

Cholestanol-3α Sulfite.—This compound was prepared in the same manner as the 3β isomer. When the reaction product was digested with ethanol, the cholestanol-3α sulfite was separated as the insoluble fraction. Recrystallization from ethyl acetate gave tiny rods, m. p. 210.5–211.5°. After drying *in vacuo* at 100° overnight the m. p. was lowered to 204–205°; $[\alpha]^{31.5}D$ +39.0 ± 2° (chloroform).

Anal. Calcd. for $C_{54}H_{94}O_3S$: C, 78.77; H, 11.51; S, 3.89. Found: C, 79.00; H, 11.28; S, 4.08.

Acid Hydrolysis of Sodium Cholestanol-3α Sulfate.—A suspension of 150 mg. of sodium cholestanol-3α sulfate in 50 cc. of 0.2 *N* hydrochloric acid solution (*p*H 0.9) was extracted with ether in a continuous extractor for forty-eight hours at room temperature. The ether extract was washed with sodium carbonate solution and water, dried over sodium sulfate, and then evaporated to dryness yielding a crystalline residue weighing 116 mg. (97% of the theoretical yield).

The crystalline residue was dissolved in 8 cc. of absolute ethanol and a solution of 100 mg. of digitonin in 2 cc. of 50% ethanol was added. The mixture was allowed to stand overnight at room temperature during which time the digitonide precipitated. About 100 cc. of anhydrous ether was added, the suspension was centrifuged and the supernatant ether was carefully decanted. This process was repeated several times with fresh portions of ether. The ether extracts were combined, washed with small portions of water and dried. Evaporation of the ether left 96 mg. (81%) of cholestanol-3α, m. p. 183–187°. Recrystallization from acetone gave a sample melting at 185–187°; $[\alpha]^{28}D$ +30.0 ± 2° (ethanol); reported[18] $(\alpha)D$ +33.9°, and which did not depress the m. p. of an authentic sample of cholestanol-3α, m. p. 186–187°. Cholestanol-3α acetate was prepared in the usual way, m. p. 94–95.5° (methanol); reported[19] m. p. 95–96°.

The ether insoluble digitonide was dissolved in 5 cc. of pyridine and heated on a steam-bath for one hour. After cooling to room temperature, 100 cc. of anhydrous ether was added to precipitate the digitonin. The suspension was centrifuged, the supernatant carefully decanted, and the process repeated with fresh portions of ether. The ether fractions were combined, washed with 10% sulfuric acid solution and water, and dried over sodium sulfate. Upon evaporation of the ether, 19 mg. (16%) of a crystalline residue, m. p. 175–182° with softening at 150°, was obtained. Recrystallization from acetone gave 12 mg. of cholestanol-3α identified by its infrared spectrum and m. p. 185–186°. The presence of cholestanol-3α in the β-hydroxy steroid fraction was not unexpected since Noller[20] has shown that α-hydroxy as well as β-hydroxy steroids may form insoluble digitonides.

The oily material (7 mg.) remaining in the mother liquor from the above recrystallization was submitted to infrared spectroscopy. The spectrum obtained showed absorption characteristic of cholestanol-3α in the region 1185–875 cm.$^{-1}$. In Figure 1 are shown the infrared absorptions of cholestanol-3α and cholestanol-3β in the region of 1185–875 cm.$^{-1}$. These curves are the tracings obtained directly from the automatic recording instrument. They illustrate the relative characteristic absorption of these compounds in this region of the infrared and they demonstrate how these tracings can be used for the rapid detection and identification of compounds with-

(15) The melting points were determined in a Hershberg melting point apparatus and are correct to about ±1°. The analyses were done by Dr. A. Elek, Rockefeller Institute for Medical Research, and Mr. J. Alicino, Metuchen, N. J.

(16) We wish to express our gratitude to Dr. K. Dobriner and Mrs. P. Humphries for their help in determining and interpreting the infrared spectra reported herein.

(17) Sobel and Spoerri, THIS JOURNAL, **63**, 1259 (1941).

(18) Windaus and Uibrig, *Ber.*, **47**, 2384 (1924).

(19) Ruzicka, Bruengger, Eichenberger and Meyer, *Helv. Chim. Acta*, **17**, 1407 (1934).

(20) Noller, THIS JOURNAL, **61**, 2717 (1939).

out the necessity of establishing the per cent. transmission curves.[21]

In this experiment 91% (100 mg.) of crystalline cholestanol-3α was obtained from its sulfate by hydrolysis. If any isomeric cholestanol-3β was present in the residue from the mother liquor of the β-hydroxy fraction, it was in too small an amount to be detected by infrared analysis.

Acid Hydrolysis of Sodium Cholestanol-3β Sulfate.—A suspension of 200 mg. of sodium cholestanol-3β sulfate in 50 cc. of 0.2 *N* hydrochloric acid solution (*p*H 0.9) was extracted in a continuous extractor for nineteen hours at room temperature. The ether extract was washed with sodium carbonate solution and water and dried over sodium sulfate. Evaporation of the solvent yielded 156 mg. (98% of the theoretical). This material was recrystallized from acetone (113 mg.), m. p. 143–143.5°, reported[22] 141–142°; $[\alpha]^{29}D$ +33.2 ± 5°; reported[18] $[\alpha]D$ +28.8°; admixture with pure cholestanol-3β did not depress the melting point. Cholestanol-3β acetate was prepared in the usual way. After recrystallization from methanol, it melted at 108–110°; reported[22] m. p. 110–111°; the admixture with an authentic sample did not depress the melting point.

The mother liquor from the recrystallization of the cholestanol-3β was concentrated to dryness and the residue (43 mg.) was separated by digitonin as described above. The crystalline β-hydroxy steroid fraction weighed 26 mg. Recrystallization from acetone gave cholestanol-3β, m. p. 140.5–141°. Infrared analysis of the material remaining in the mother liquor demonstrated the presence of cholestanol-3β; no α-isomer was indicated. The non-crystalline α-hydroxysteroid fraction weighed 11 mg. and showed no characteristic absorption between 1185 and 875 cm.$^{-1}$. The presence of cholestanol-3α in quantities greater than 1 mg. would have been detected in this fraction by this method.

Eighty-eight per cent of crystalline cholestanol-3β was obtained by acid hydrolysis of its sulfate.

Acid Hydrolysis of Sodium Cholesterol Sulfate.—A suspension of 150 mg. of sodium cholesterol sulfate in 50 cc. of 0.2 *N* hydrochloric acid was extracted with ether in a continuous extractor for forty-eight hours at room temperature. The ether extract was worked up in the same manner as that used for sodium cholestanol-3α sulfate. The residue from the ether extract weighed 106 mg. (98% based on hydrated sulfate). After digitonin separation the crystalline β-hydroxy fraction weighed 93 mg. (86%). Recrystallization from acetone gave a sample, m. p. 147–148.5°; $[\alpha]^{22}D$ −30.0 ± 2° (ethanol); which did not depress the melting point of an authentic sample of cholesterol; reported[23] $[\alpha]D$ −29.9° (ethanol). The acetate melted at 114.5–115.5° and did not depress the m. p. of an authentic sample.

The α-hydroxy fraction (10 mg.) was a brown oil which did not crystallize. Infrared analysis indicated no characteristic absorption in the region 1185–875 cm.$^{-1}$.

Acid Hydrolysis of Potassium Dehydroisoandrosterone Sulfate.—By the foregoing procedure 150 mg. of potassium dehydroisoandrosterone sulfate was hydrolyzed. The hydrolysate weighed 96 mg. (94% based on hydrated sulfate) and was separated by digitonin. The β-hydroxysteroid fraction weighed 87 mg. (85%) and after recrystallization from acetone, the product melted at 146–148°; $[\alpha]^{23}D$ +12.5 ± 2° (ethanol); reported[24] 148–149°; $[\alpha]D$ +10.9° (ethanol)[25]; the admixture with dehydroisoandrosterone melted at 145–147°. The acetate melted at 168–170°; reported[26] 170–171°.

The α-hydroxysteroid fraction weighed 8 mg. and was not crystalline. It showed no characteristic absorption in the 1185–875 cm.$^{-1}$ region of the infrared spectrum.

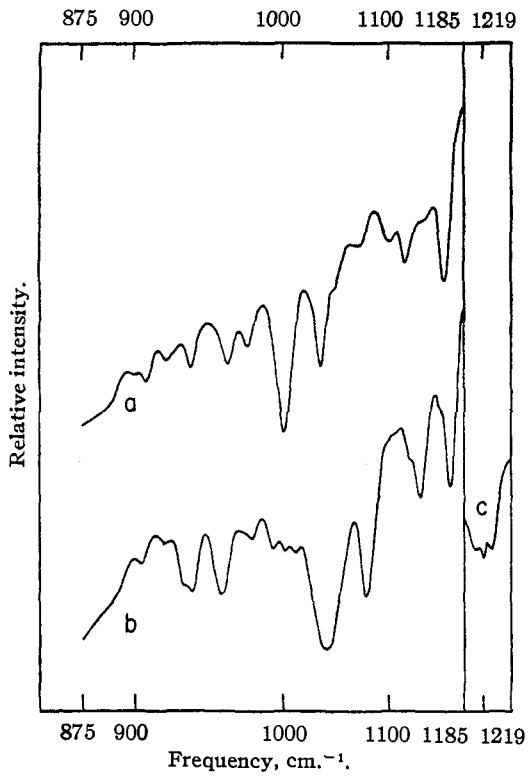


Fig. 1.—Infrared tracings of approximately 1% carbon disulfide solutions of (a) cholestanol-3α and (b) cholestanol-3β taken in a 1-mm. cell. Curve C shows some characteristic absorption bands of acetone vapor which is used as an external standard.

Reaction of Sodium Cholestanol-3α Sulfate with Silver Acetate.—About 20 cc. of anhydrous acetic acid (distilled over triacetyl borate)[27] was distilled into a flask containing 100 mg. of sodium cholestanol-3α sulfate and 200 mg. of silver acetate and the mixture was refluxed for four hours. The acetic acid was distilled *in vacuo* and the residue was extracted several times with ether. The ether extract was washed with sodium carbonate solution, water and dried over sodium sulfate. The oily residue (75 mg.) was dissolved in 7 cc. of ligroin and chromatographed on 2 g. of alumina.[28] Three fractions were obtained: (1) 2 mg. of oil eluted by ligroin and identified by infrared analysis as neocholestene. (2) 51 mg. of crystalline cholestanol-3α acetate, eluted with ligroin. Recrystallization from methanol–acetone gave needles melting at 95.5–96°, which did not depress the melting point of an authentic sample. Infrared analysis of the mother liquor showed the presence of cholestanol-3α acetate, but did not indicate any cholestanol-3β acetate. (3) 22 mg. of crystalline cholestanol-3α after recrystallization from methanol–acetone melting at 186–187°.

(21) Dobriner, Lieberman, Rhoads, Jones, Williams and Barnes, *J. Biol. Chem.*, **172**, 297 (1948).

(22) Willstätter and Mayer, *Ber.*, **41**, 2199 (1908).

(23) Mauthner, *Monatsh.*, **27**, 421 (1906).

(24) Wolfe, Fieser and Friedgood, THIS JOURNAL, **63**, 582 (1941).

(25) Butenandt, Dannenbaum, Hanisch and Kudszus, *Z. physiol. Chem.*, **237**, 57 (1935).

(26) Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 986 (1935).

(27) Eichelberger and LaMer, THIS JOURNAL, **55**, 3633 (1933). This experiment was repeated with acetic acid which was dried simply by freezing and decantation and the results were essentially the same except that more neocholestene was formed. The neocholestene was identified by m. p. (70–71°) and infrared analysis.

(28) This alumina was specially prepared and kindly made available to us by Dr. T. F. Gallagher [Hollander and Gallagher, *J. Biol. Chem.*, **162**, 549 (1946)]. It has been observed that 3-acetoxysteroids are not appreciably hydrolyzed when chromatographed on this acetic acid-washed alumina.

Infrared analysis of the residue from the mother liquor did not indicate any cholestanol-3β.

The total amount of cholestanol-3α corresponds to 68 mg. (46 mg., as the acetate and 22 mg. as the free alcohol) or 86%.

When cholestanol-3α acetate was heated in acetic acid in the presence of silver acetate, cholestanol-3α acetate was recovered unchanged; cholestanol-3α under the same conditions yielded 62% cholestanol-3α acetate and 38% cholestanol-3α.

Reaction of Sodium Cholestanol-3β Sulfate with Silver Acetate.—A suspension of 100 mg. of sodium cholestanol-3β sulfate, 200 mg. of silver acetate and 20 cc. of acetic acid (distilled over triacetyl borate) was refluxed for four hours. The reaction mixture was worked up as above and yielded on chromatographic analysis: (1) 39 mg. of crystalline cholestanol-3β acetate. After recrystallization from methanol–acetone, 29 mg., m. p. 109–110°, was obtained. The residue in the mother liquor was shown by infrared analysis to be cholestanol-3β acetate. (2) 28 mg. of crystalline cholestanol-3β. After recrystallization from methanol–acetone, it weighed 18 mg., m. p. 140–142°. Infrared analysis of the mother liquor again indicated the presence of only the β isomer. The amount of cholestanol-3β recovered was 63 mg. (35 mg. as the acetate and 28 mg. as the alcohol) or 80%.

Reaction of Sodium Cholestanol-3α Sulfate in Methanolic Hydrogen Chloride.—Dry hydrogen chloride gas was bubbled through a suspension of 185 mg. of sodium cholestanol-3α sulfate in 20 cc. of absolute methanol for five minutes. The sulfate dissolved immediately but after the solution remained overnight at room temperature, a precipitate had formed. The product (101 mg., 69%) melted at 186–186.5°, and when mixed with an authentic sample of cholestanol-3α, the melting point was not depressed.

The filtrate was concentrated to dryness and extracted with ether. The ether extract was washed with sodium carbonate solution and water, dried and the solvent removed by distillation under reduced pressure. The residue (31 mg.), m. p. 175–183°, was separated by digitonin. The α-hydroxy fraction (16 mg., 11%) was crystalline cholestanol-3α, m. p. 185–187°. The digitonide was dissociated and yielded 11 mg., m. p. 165–178°. Crystallization from acetone gave cholestanol-3α, m. p. 185–186°. The residue (2 mg.) from the mother liquor had an infrared spectrum characteristic of cholestanol-3α and there was no absorption characteristic of cholestanol-3β. In this experiment 126 mg. (86%) of crystalline cholestanol-3α was obtained.

Reaction of Sodium Cholestanol-3β Sulfate with Methanolic Hydrogen Chloride.—Sodium cholestanol-3β sulfate (200 mg.) was suspended in 20 cc. of absolute methanol into which dry hydrogen chloride gas had been bubbled for five minutes. The mixture was allowed to stand overnight at room temperature and the solvent removed by distillation under reduced pressure. The residue was extracted with ether, the ether solution was washed with sodium carbonate solution and water, and dried. Evaporation of the solvent yielded 144 mg. which was recrystallized from acetone. Sixty-six mg. (42%), m. p. 142–142.5° was obtained which upon mixing with cholestanol-3β did not depress the melting point. The residue (78 mg.) from the crystalline product was separated by digitonin. The β-hydroxy fraction (69 mg., 44%) was crystalline and upon crystallization from acetone gave cholestanol-3β, m. p. 140–142°. The residue (4 mg.) showed absorption bands in the infrared characteristic of cholestanol-3β. The non-crystalline α-hydroxysteroid fraction weighed 7 mg. and exhibited no characteristic absorption in the 1185–875 cm.$^{-1}$ region. The total yield of crystalline cholestanol-3β was 86%.

Reaction of Cholestanol-3α Sulfite with Silver Acetate.—A mixture of 150 mg. of cholestanol-3α sulfite with 200 mg. of silver acetate was refluxed seven hours in 45 cc. of acetic acid (dried by freezing and decantation). After working up in the usual way, 151 mg. of an ether soluble oil, was obtained. It was separated by chromatographic analysis on 4.6 g. of alumina and yielded 14 mg. of neocholestene, 70 mg. of crystalline cholestanol-3α acetate (recrystallized from acetone, 55 mg., m. p. 87–91°), 12 mg. of unreacted sulfite (eluted with benzene–ligroin (1:1)), and 42 mg. crystalline cholestanol-3α (recrystallized from methanol–acetone, 26 mg., m. p. 186.5–187°). The cholestanol-3α recovered amounted to 106 mg. (64 mg. as the acetate and 42 mg. as the alcohol) or 84% based on the sulfite which had reacted.

The compounds were identified by melting point and infrared spectral analysis. The infrared analysis of the residues showed that only cholestanol-3α and its acetate were present. Neither cholestanol-3β nor its acetate was detected.

Reaction of Cholestanol-3β Sulfite with Silver Acetate.—A mixture of 150 mg. of cholestanol-3β sulfite, 200 mg. of silver acetate and 45 cc. of anhydrous acetic acid was refluxed for four hours. The reaction mixture was worked up in the usual way and yielded on chromatographic analysis: (1) 2 mg. of neocholestene; (2) 11 mg. of unreacted sulfite; (3) 58 mg. of crystalline cholestanol-3β acetate which melted at 107–109.5° after a recrystallization from methanol–acetone. The infrared analysis of the mother liquor indicated the presence of only cholestanol-3β acetate; (4) 58 mg. of crystalline cholestanol-3β. After recrystallization from methanol, 43 mg., m. p. 140–142° was obtained. The residue in the mother liquor was shown by infrared analysis to be cholestanol-3β.

Reaction of Cholestanol-3α Sulfite with Methanolic Hydrogen Chloride.—Dry hydrogen chloride was bubbled through 20 cc. of absolute methanol for five minutes. After the solution had cooled to room temperature, 100 mg. of cholestanol-3α sulfite was added and the suspension allowed to stand at room temperature for twenty-four hours. The reaction product was worked up in the usual way and chromatographed on alumina. The fractions obtained were: 4 mg. of an oil eluted with ligroin whose spectra could not be identified, 15 mg. of unreacted sulfite, and 64 mg. (80% based on reacted sulfite) of crystalline cholestanol-3α, m. p. 181–185°.

Reaction of Cholestanol-3β Sulfite with Methanolic Hydrogen Chloride.—Cholestanol-3β sulfite (100 mg.) was treated with methanolic hydrogen chloride as above. The reaction product was worked up in the usual way and chromatographed on alumina. Twenty-four milligrams of cholestanol-3β sulfite, and 69 mg. (95% based on reacted sulfite) of cholestanol-3β were obtained. Recrystallization from methanol–acetone gave 60 mg. of m. p. 141–142°. The infrared analysis of the mother liquor from the recrystallization of cholestanol-3β showed the presence of only the β-isomer.

Summary

The acid sulfates and the sulfites of several steroids have been prepared and their cleavage has been studied. The reaction of these compounds with aqueous acid solution, dry hydrogen chloride in methanol, or silver acetate in acetic acid proceeds with retention of configuration indicating that these compounds react by a rupture of the S-OR linkage. These results are of biological importance because they demonstrate that the steroid acid sulfates excreted in the urine are hydrolyzed without Walden inversion.

NEW YORK, N. Y. RECEIVED NOVEMBER 14, 1947