ture resulted in the isolation of 0.56 g. (70%) of 4-chlorothiophenol, m. p. 51.5–53°, and a neutral oil which was similar to that isolated from the cleavage of benzyl phenyl sulfide.

Summary

1. 4-Bromothiophenol has been found to react rapidly with toluene, chlorobenzene and benzene in the presence of aluminum bromide, to form 4-methyl-, 4-chloro- and unsubstituted diphenyl sulfide, respectively, with loss of hydrogen bromide. 2-Bromothiophenol behaves similarly, but the 3-bromo and the 4-chloro compounds do not give this reaction. Numerous kinetic and other experiments on the reaction are reported, leading to a proposed mechanism.

2. The rate of cleavage of benzyl 4-chlorophenyl and 3-bromophenyl sulfides by aluminum bromide has been measured, and the ρ value (Hammett) for the reaction has been determined.

3. Numerous new compounds incidental to the work have been prepared and characterized.

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

Preparation of Deuterated Steroids[1]

BY DAVID K. FUKUSHIMA, SEYMOUR LIEBERMAN AND BERNADETTE PRAETZ

In recent years there has been increasing recognition of the important role steroid hormones play in the function and regulation of various physiological processes. Because of the extraordinary success of the isotope tracing technique in clarifying other biochemical and physiological problems, there is also great interest in the application of this tool to the problems of steroid biochemistry.[2] With this in mind, two methods for the incorporation of deuterium atoms on C_7 of Δ^5-unsaturated steroids have been developed. These methods are general and can be applied to a variety of steroid hormones or related compounds. They possess the advantage that the deuterium is introduced in a known and stable position in compounds which still retain the 5,6-double bond and which therefore may readily be converted to α,β-unsaturated 3-ketones. Moreover, another desirable property of the deuterated steroids is that the presence of a carbon–deuterium bond in the steroid molecule can be detected easily by its specific absorption maximum in the infrared at about 2100 cm.$^{-1}$. This property not only facilitates the isolation of deuterated steroids, but also provides a method for the semiquantitative analysis of the deuterium content of isotopically labeled substances.

The report of Henbest, *et al.*,[3] on the preparation of 7-bromocholesterol acetate by N-bromosuccinimide bromination of cholesterol acetate suggested to us the possibility of employing such Δ^5-7-bromo-derivatives as intermediates. As illustrated in Fig. 1, the replacement of the halogen of these derivatives (II) with deuterium affords tagged steroids containing the isotope in the stable position on C_7.

The procedure described[4] for the allylic bromination of cholesterol acetate with N-bromosuccinimide yielded in our hands an uncrystallizable oil[5] which, after chromatography on magnesium silicate:Celite, afforded 3β-acetoxy-Δ^5-cholestene-4β,7α-diol, a compound which will be described in detail in the following paper.[6] For our deuteration experiments, therefore, we have employed 7-bromocholesterol benzoate which can easily be obtained crystalline.[7] We have also brominated Δ^5-androstene-3β,17β-diol 3-acetate-17-benzoate (Ib) with N-bromosuccinimide and obtained a crystalline 7-bromo-derivative (IIb) in 40% yield. The position of the bromo group in IIb was proved by replacing the halogen with hydroxyl on an alumina column and oxidizing the resulting alcohol to the 7-keto derivative, 3β-acetoxy-17β-benzoxy-Δ^5-androsten-7-one (IV). This 7-keto compound was also prepared by the direct chromic acid oxidation of Ib, following the method of Windaus, Lettré and Schenck.[8]

A variety of methods for the reduction of the 7-bromo group of these derivatives was explored. Reduction of 7-bromocholesterol benzoate (IIa) with deuterium was best accomplished by using "deuterized" Raney nickel in ethyl acetate to give 63% of 7-*d*-cholesterol containing 1.55 atom per cent. excess deuterium. 7-Bromo-Δ^5-androstene-

(1) Presented before the 33rd Meeting of the Federation of American Societies for Experimental Biology, Detroit, Michigan, March, 1949; *Fed. Proc.*, **8**, 200 (1949).

(2) A number of isotopically labeled steroids have already been reported: (a) cholestenone and testosterone with C^{14} in ring A, Turner, *Science*, **106**, 248 (1948); THIS JOURNAL, **69**, 726 (1947); (b) C^{14}-methyl-labeled methyltestosterone, MacPhillamy and Scholz, *J. Biol. Chem.*, **178**, 37 (1949); (c) C^{14}-methyl-labeled progesterone, Riegel and Prout, *J. Org. Chem.*, **13**, 933 (1938); MacPhillamy and Scholz, *J. Biol. Chem.*, **178**, 37 (1949); (d) dehydroisoandrosterone with C^{13} in ring D, Hershberg, Schwenk and Stahl, *Arch. Biochemistry*, **19**, 300 (1948); (e) deuterated cholesterol, Bloch and Rittenberg, *J. Biol Chem.*, **149**, 505 (1943); Anker, THIS JOURNAL, **66**, 1752 (1944); (f) progesterone and testoterone with deuterium at C_{11} and C_{12}, Koechlin, Kritchevsky and Gallagher, *J. Biol. Chem.*, **184**, 393 (1950).

(3) Henbest, Jones, Bide, Peevers and Wilkinson, *Nature*, **158**, 169 (1946).

(4) British Patent 574,432.

(5) In a more recent publication, the isolation of crystalline 7-bromocholesterol acetate has been reported in detail: Bide, Henbest, Jones, Peevers and Wilkinson, *J. Chem. Soc.*, 1783 (1948).

(6) Lieberman and Fukushima, THIS JOURNAL, **72**, 5211 (1950).

(7) Buisman, Stevens and van der Vliet, *Rec. trav. chim.*, **66**, 83 (1947); Bernstein, *et al.*, *J. Org. Chem.*, **14**, 433 (1949).

(8) Windaus, Lettré and Schenck, *Ann.*, **520**, 98 (1935).

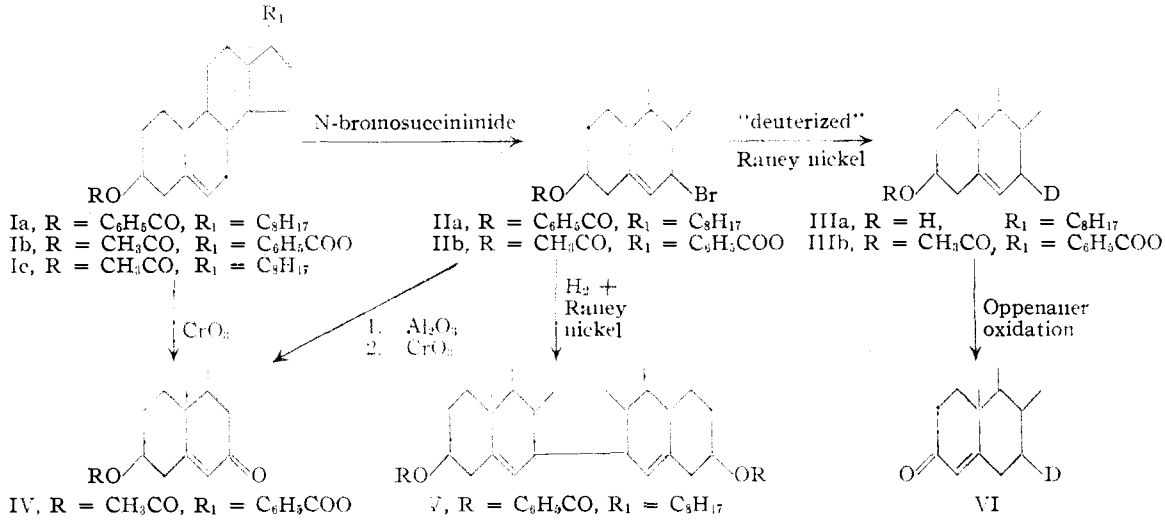


Fig. 1.

3β,17β-diol 3-acetate-17-benzoate (IIb) was reduced in 49% yield with "deuterized" Raney nickel to the corresponding deuterated derivative (IIIb) containing 1.18 atom per cent. excess deuterium. The catalytic reduction of the bromide IIa with 5% palladium on calcium carbonate gave 52% of 7-*d*-cholesterol containing 1.39 atom per cent. excess deuterium. The reduction of the bromides by these catalysts was accompanied by coupling[9] and in the case of IIa, resulted in 7,7'-bicholesterol benzoate (V). A high ratio of Raney nickel to the bromide was found to be most effective in minimizing the formation of the coupled product. Other solvents such as cyclohexane and ethanol were employed in the reduction with or without addition of bases such as sodium hydroxide, pyridine or triethylamine, but the yield of cholesterol was not improved. Reduction with zinc or zinc–copper couple in acetic acid gave large amounts of the coupled derivative and only poor yields of cholesterol. Several attempts were made to reduce 7-bromocholesterol benzoate with lithium aluminum deuteride,[10] however, the reduction did not proceed as expected. Although its presence was indicated by the Liebermann–Burchard determination, no cholesterol could be easily isolated from the reaction mixture.

That the deuterium atom on C_7 is stably bound was shown by the fact that no isotope was lost when 7-*d*-cholesterol benzoate was saponified to 7-*d*-cholesterol. Furthermore the isotope concentration was unchanged when 7-*d*-cholesterol (IIIa) was oxidized to 7-*d*-Δ^4-cholesten-3-one (VI). Refluxing the α,β-unsaturated ketone (VI) with methanolic potassium hydroxide for two hours did not result in any exchange of deuterium.

The second method for introducing deuterium into the steroid nucleus is illustrated in Fig. 2. This procedure is essentially that of Wolfrom and Karabinos[11] who first prepared a thioacetal from a ketone for the express purpose of desulfurizing it with Raney nickel[12] in order to convert a carbonyl to a methylene group. It is therefore possible to introduce deuterium into the steroid nucleus by the desulfurization of appropriate steroid thioacetals[13] with "deuterized" Raney nickel. This procedure results in steroids which theoretically should contain two deuterium atoms in the molecule. It is apparent from the isotopic content of the products that the deuterium introduced was less than calculated for two deuterium atoms and the 7,7-d_2-nomenclature is used with this fact in mind. Δ^5-7-Keto-compounds, easily prepared by the direct chromic acid oxidation of the Δ^5-unsaturated steroids, appeared to be most suitable for the preparation of steroid hormones with the essential functional groups at C_3, C_{17} or C_{20}. The 7-keto derivatives (VII) were also prepared by way of the 7-bromo compounds as was mentioned above.

The same steroids, cholesterol and Δ^5-androstene-3β-17β-diol 3-acetate-17-benzoate, which were used to prepare the 7-bromo intermediates, were converted by chromic acid oxidation to their 7-keto derivatives. These Δ^5-7-ketones (VIIa and VIIb) readily form cyclic mercaptols (VIIIa and VIIIb) with ethanedithiol in the presence of freshly fused zinc chloride as Haupt-

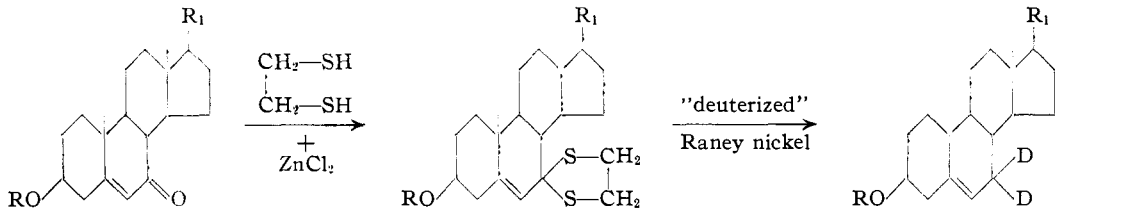
(9) Coupling reactions occurring during catalytic reduction of halogenated compounds have been extensively studied: Busch and Weber, *J. prakt. Chem.*, **146**, 1 (1936); Busch, *Angew. Chem.*, **38**, 518 (1925); **47**, 536 (1934); Grigorovskii and Fedorov, *Zhur. Prikład. Khim.*, **21**, 529 (1948) (*C. A.*, **43**, 646 (1949)).

(10) Nystrom and Brown, THIS JOURNAL, **70**, 3738 (1948); Johnson, Blizzard and Carhart, *ibid.*, **70**, 3664 (1948).

(11) Wolfrom and Karabinos, *ibid.*, **66**, 909 (1944).

(12) Bougault, Cattelain and Chabrier, *Compt. rend.*, **208**, 657 (1939); *Bull. soc. chim.*, [5] **6**, 341 (1939); **7**, 781 (1940); Mozingo, Wolf, Harris and Folkers, THIS JOURNAL, **65**, 1013 (1943).

(13) Recently the preparation of a number of steroid thioacetals has been reported: (a) Bernstein and Dorfman, *ibid.*, **68**, 1152 (1946); (b) Hauptmann, *ibid.*, **69**, 562 (1947); (c) Norymberska, Norymberski and Olalde, *ibid.*, **70**, 1256 (1948).



VII, R = CH_3CO, R_1 = C_8H_{17}
VIIb, R = CH_3CO, R_1 = C_6H_5COO

VIIIa, R = CH_3CO, R_1 = C_8H_{17}
VIIIb, R = CH_3CO, R_1 = C_6H_5COO
VIIIc, R = C_6H_5CO, R_1 = C_8H_{17}
VIIId, R = H, R_1 = C_8H_{17}

IXa, R = H, R_1 = C_8H_{17}
IXb, R = CH_3CO, R_1 = C_6H_5COO

Fig. 2.

mann[13b] has shown with saturated 7-keto and Δ^4-unsaturated-3-ketosteroids.[14] The ethylene mercaptols of the Δ^5-7-ketones were desulfurized with "deuterized" Raney nickel to afford the corresponding 7,7-d_2-Δ^5-derivatives (IXa and IXb) in 50–60% yield. 7,7-d_2-Cholesterol prepared in this manner contained 2.09 atom per cent. excess deuterium and 7,7-d_2-Δ^5-androstene-3β,17β-diol 3-acetate-17-benzoate contained 4.00 atom per cent. excess deuterium. That deuterium atoms had exchanged with some of the hydrogen atoms of the ester groups of this latter compound during desulfurization was shown by the fact that the Δ^5-androstenediol obtained after hydrolysis contained only 2.15 atom per cent. excess deuterium. In the reduction of the bromo derivatives (II) with "deuterized" Raney nickel, little or no exchange was encountered because the saponified products had the same isotope concentration as the esters.

In all the deuteration experiments cited above, the amount of deuterium introduced into the steroid nucleus is less than that which would be expected from stoichiometric considerations alone. This is due to an isotope fractionation in favor of hydrogen which was present in both of the methods described above. In the method involving the catalytic reduction of the 7-bromo steroids, the hydrogen arises from the deuterium gas which contained about 5 per cent. of the lighter element. Furthermore, the catalysts, palladium on calcium carbonate and "deuterized" Raney nickel, employed in this method and in the desulfurization experiments still contained small but significant amounts of hydrogen. Thus, even though the hydrogen is present in only relatively small amounts, it reacts at a much faster rate than does deuterium and this results in the introduction of less deuterium than the ratio of deuterium to hydrogen would lead one to anticipate.

The Ultraviolet Absorption Spectra of Steroid Mercaptols.—During the course of this investigation, a number of papers by Fehnel and Carmack[15] and by Koch[16] appeared which discussed the absorption spectra of various organic sulfur compounds. Since the steroid mercaptols synthesized in the present work were structurally different from any of the compounds employed by these investigators, we have determined their spectra in the region between 215–320 mμ and the maxima are tabulated in Table I. If proper

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF STEROID MERCAPTOLS

Compounds	Max λmμ	ϵ_λ	ϵ_λ desthio derivative	$\Delta\epsilon$
7-Ketocholesterol acetate ethylene mercaptol (VIIIa)	222	6,430	80[a]	6350
7-Ketocholesterol benzoate ethylene mercaptol (VIIIc)	230	19,400	14,400[b]	5000
3β-Acetoxy-17β-benzoxy-Δ^5-androsten-7-one ethylene mercaptol (VIIIb)	228	20,800	14,000[c]	6800
Cholestan-3-one ethylene mercaptol (X)	240	333		
2,2-bis-(*n*-butylmercapto)-propane (XI)	237	660[15]		

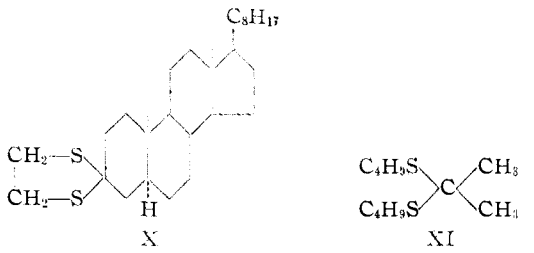
[a] Cholesterol acetate (end absorption). [b] Cholesterol benzoate (ϵ_{max} = 14,400 at 230 mμ). [c] Δ^5-Androstene-3β,17β-diol 3-acetate-17-benzoate (ϵ_{max} = 14,100 at 230 mμ).

allowances are made for the absorption of the acetate and benzoate groups, the contribution of the cyclic $\Delta^{5,6}$-unsaturated mercaptols to the absorption spectra of compounds VIIIa, VIIIb and VIIIc is found to be about 6,000 ($\Delta\epsilon$). This is greater than that (333) of the cyclic saturated steroid mercaptol, cholestan-3-one ethylene mercaptol (X). This latter value agrees fairly well with the value (660) obtained by Fehnel and Carmack for the simple acyclic mercaptol, 2,2-bis-(*n*-butylmercapto)-propane (XI). The higher extinction coefficient of the unsaturated steroid mercaptols must therefore be related to the presence of the 5,6-double bond even though it might be expected that the effect of this unsaturation would be insulated from the chromophoric group by the quaternary carbon of the spirane ring. That the double bond is not in true

(14) In a recent publication Ralls, Dodson and Riegel (THIS JOURNAL, **71**, 3320 (1949)) prepared and desulfurized VIIIa in order to reduce the carbonyl group in 7-ketocholesterol acetate.

(15) Fehnel and Carmack, *ibid.*, **71**, 84 (1949).

(16) Koch, *J. Chem. Soc.*, 387 (1949).



X XI

Fig. 3.

conjugation with the chromophore is evidenced by the fact that the wave length of the maxima of the unsaturated mercaptols is approximately the same as that of the saturated derivative.

Acknowledgments.—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.

Experimental[17]

"Deuterized" Raney Nickel.[18]—The Raney nickel catalyst (W-4) was prepared by the method of Pavlic and Adkins[19] and was found to contain about 165 cc. of hydrogen per cc. of catalyst (equivalent to about 0.7 g. of dry nickel). To 100 cc. of Raney nickel catalyst (under ethanol) was added 800 cc. of methylcyclohexane. The alcohol and any water present were removed by azeotropic distillation with methylcyclohexane at 70° (bath temperature) at 20 cm. To the dry catalyst was added 13.5 cc. of deuterium oxide (99.9 per cent.) and 1 l. of dry methylcyclohexane. The mixture was shaken well and warmed for fifteen minutes. The equilibrated deuterium oxide was removed by azeotropic distillation with methylcyclohexane. This equilibration was repeated five times with volumes of 13.5, 12.5, 12, 12 and 11.5 cc. of deuterium oxide. The water from the last equilibration contained 88.5% deuterium oxide. Dry methylcyclohexane was added at each equilibration to replace the amount distilled. After the equilibration was complete, the "deuterized" Raney nickel was shaken with deuterium gas (95%).

7-*d*-Cholesterol (IIIa) from IIa with "Deuterized" Raney Nickel as Catalyst.—To a suspension of 20 cc. of "deuterized" Raney nickel was added 2.00 g. of 7-bromocholesterol benzoate[7,20] (IIa) in 250 cc. of dry ethyl acetate. The mixture was shaken with deuterium (95%) under atmospheric pressure for one hour. The catalyst was removed by decantation and filtration with the aid of Celite. Because a considerable amount of the product remained adsorbed on the nickel, it was necessary to wash the catalyst several times with ethyl acetate to insure a quantitative recovery. The solvent was evaporated and the residue was hydrolyzed with 50 cc. of benzene, 125 cc. of methanol and a solution of 5 g. of potassium hydroxide in 5 cc. of water. After refluxing one hour the solvents were removed *in vacuo*, water was added and the residue extracted with ether. The ether solution was washed with water, dried over sodium sulfate and evaporated to dryness. The semi-crystalline residue, 1.68 g., was chromatographed on 30 g. of alumina. The column was developed with ligroin (b. p. 30°), benzene: ligroin (1:1), benzene and mixtures of ether and benzene, and mixtures of methanol and ether. From the benzene and benzene–ether (3:1) eluates was obtained 960 mg. of crude 7-*d*-cholesterol, which upon recrystallization from acetone gave 592 mg. of pure 7-*d*-cholesterol, m. p. 148–149°; $[\alpha]^{25}D$ −40.5° ± 2° (11.36 mg. in 2.00 ml. of chloroform); the m. p. of the admixture with undeuterated cholesterol was not depressed. From the mother liquor was obtained 250 mg. of 7-*d*-cholesterol with m. p. 147.5–148.5°, giving a total of 844 mg. (63%) of 7-*d*-cholesterol. Infrared analysis of these samples revealed the presence of the characteristic absorption at 2100 $cm.^{-1}$ associated with the C–D bond. Quantitative analysis with the mass spectrometer indicated that the 7-*d*-cholesterol contained 1.55 atom per cent. excess deuterium, corresponding to 0.71 atom deuterium per molecule.

From another reduction of 7-bromocholesterol benzoate with "deuterized" Raney nickel (which was prepared by four equilibrations with deuterium oxide), 7-*d*-cholesterol benzoate, m. p. 144–146°, clear at 180°, was obtained by recrystallization of the reaction mixture from acetone. This sample contained 0.94 atom per cent. excess deuterium, corresponding to 0.47 atom deuterium per molecule. 7-*d*-Cholesterol (m. p. 148–148.5°; $[\alpha]^{29}D$ −39.4 ± 4° (chloroform)) obtained by the hydrolysis of this ester contained 1.04 atom per cent. excess deuterium or 0.48 atom deuterium per molecule. This indicates that no exchange with the hydrogen of the ester group had occurred and also indicates that under the conditions of saponification, the deuterium on C_7 is stably bound.

7-*d*-Cholesterol (IIIa) from IIa Using Palladium as Catalyst.—Five grams of 5% palladium on calcium carbonate was prereduced with deuterium in 25 cc. of dry ethyl acetate. One-half gram of 7-bromocholesterol benzoate (IIa) in 35 cc. of dry ethyl acetate was added and the mixture shaken with deuterium for one and one-half hours. The reduction mixture was worked up and hydrolyzed as above to give 275 mg. of crystalline material. Purification by chromatography on acid-washed alumina yielded 178 mg. (52.5%) of crystalline 7-*d*-cholesterol in the benzene eluate. After recrystallization from acetone, it melted at 145.5–147°; $[\alpha]^{25}D$ −40.8° ± 3° (5.82 mg. in 2.00 ml. of chloroform). The deuterium content of this sample was 1.39 atom per cent. excess deuterium, corresponding to 0.64 atom deuterium per molecule.

Reduction of IIa with Lithium Aluminum Deuteride.—One gram of 7-bromocholesterol benzoate (IIa) was placed in a thimble in a Soxhlet extractor, and 200 mg. of lithium aluminum deuteride in 150 cc. of dry ether was placed in the extraction flask. Upon heating, the ether extracted the benzoate from the thimble and thus slowly brought it into reaction with the lithium aluminum deuteride. After one and one-half hours the reaction mixture was cooled and water was added followed by dilute sulfuric acid. The ether solution was washed with sodium bicarbonate solution and water and dried over sodium sulfate. Evaporation of the solvent yielded 825 mg. of yellow product which gave a positive Beilstein test for halogen and smelled of benzyl alcohol.

The reaction product was hydrolyzed with methanolic potassium hydroxide solution to give 739 mg. of bromine-free product which was chromatographed on alumina. The main fraction was eluted with benzene–ligroin (1:1) and benzene and consisted of 612 mg. of white crystals. Recrystallization from acetone gave a product, m. p. 135–137°, which was estimated to contain 37% of

(17) The melting points were taken in a Hershberg apparatus and are correct to ±1°. The carbon and hydrogen analyses were done by Mr. J. Alicino, Metuchen, New Jersey. The authors wish to acknowledge their appreciation to Mr. Robert W. Jailer for the deuterium analyses reported herein and also to Dr. Harold Beyer and Mr. Mones Berman for their assistance with the mass spectrometer. We also wish to express our gratitude to Dr. K. Dobriner and Mrs. P. Humphries for the determination of the infrared spectra. For a discussion of the infrared spectra of steroids containing deuterium, see Dobriner, *et al.*, *Science*, **109**, 260 (1949).

(18) We wish to express our appreciation to Dr. R. Mozingo, Merck and Co., Rahway, New Jersey, for suggesting this method to us.

(19) Pavlic and Adkins, THIS JOURNAL, **68**, 1471 (1946).

(20) We wish to express our gratitude to Dr. Gordon Grant, Ayerst, McKenna and Harrison, Ltd., Montreal, Canada, who made a large supply of this compound available to us.

cholesterol by the Liebermann–Burchard determination. Attempts at purification of this product through the dibromide were unsuccessful; only 180 mg. of impure cholesterol, m. p. 136–137° were recovered.

7-*d*-Δ^4-Cholesten-3-one (VI).—A solution of 350 mg. of 7-*d*-cholesterol (1.04 atom per cent. excess deuterium or 0.48 atom deuterium per molecule) in 50 cc. of toluene and 8 cc. of cyclohexanone was distilled until the distillate was free of water. To the solution was then added 0.6 g. of aluminum tertiary butylate in 15 cc. of toluene. The mixture was refluxed for one hour, cooled, acidified with 10% sulfuric acid solution and was steam distilled for three and one-half hours. The residue was extracted with ether and the ether solution was washed with sodium carbonate solution and water, dried and evaporated to give 371 mg. of yellow oil. Upon chromatography on alumina, 265 mg. of crystalline compound was eluted with ligroin (b. p. 60°). Recrystallization from methanol yielded 7-*d*-Δ^4-cholesten-3-one, m. p. 79.5–80.5°, containing 1.06 atom per cent. excess deuterium or 0.47 atom deuterium per molecule.

A solution of 198 mg. of the above 7-*d*-cholestenone in 25 cc. of methanol and 15 cc. of 5% methanolic potassium hydroxide was refluxed for two hours under nitrogen. The mixture was diluted with water and extracted with ether. The ether solution was washed with water, dried and evaporated to give 198 mg. of yellow oil. Purification by chromatography on alumina and recrystallization from methanol yielded 7-*d*-cholestenone, m. p. 79–80°, containing 1.08 atom per cent. excess deuterium or 0.48 atom deuterium per molecule.

7,7′-Bicholesterol Benzoate (V).—The solvent from 8 cc. of Raney nickel was removed by distillation under reduced pressure at room temperature and replaced with 300 cc. of ethyl acetate. After the catalyst was shaken with hydrogen, 1.00 g. of 7-bromocholesterol benzoate was added. The hydrogenation was carried out at room temperature for two and one-half hours. The catalyst was removed by filtration and washed several times with benzene. The ethyl acetate filtrate and benzene washings were combined and washed with water, sodium carbonate and water. The organic extract was dried over sodium sulfate and the solvent evaporated leaving 855 mg. of semi-crystalline material. This was dissolved in 100 cc. of ligroin and one-half of it (427 mg.) was chromatographed on acid-washed alumina. Elutions with ligroin, benzene–ligroin (19:1) gave 338 mg. (78%) of cholesterol benzoate.

From the benzene–ligroin (4:1) and benzene eluates was obtained 69 mg. of 7,7′-bicholesterol benzoate (V), m. p. 194–197°. Recrystallization from acetone gave a sample melting at 194.5–196°; $[\alpha]^{26}D$ −11.7 ± 1° (17.1 mg. in 2.00 ml. of chloroform).

Anal. Calcd. for $(C_{34}H_{49}O_2)_2$: C, 83.38; H, 10.09; mol. wt., 979.5. Found: C, 83.69; H, 10.08; mol. wt., 892.

The coupled product was also obtained in the reduction of 7-bromocholesterol benzoate with zinc or zinc–copper couple and acetic acid as well as in the catalytic reduction with 5% palladium on calcium carbonate when the ratio of catalyst to bromide was low.

7-Bromo-Δ^5-androstene-3β,17β-diol-3-acetate-17-benzoate (IIb).—To a refluxing solution of 2.00 g. of Δ^5-androstene-3β,17β-diol-3-acetate-17-benzoate (Ib)[21] in 50 cc. of carbon tetrachloride were added 840 mg. of N-bromosuccinimide and 50 mg. of benzoyl peroxide. The mixture was allowed to reflux for seven minutes, then chilled and filtered. The carbon tetrachloride filtrate was washed with sodium carbonate solution and water, dried over sodium sulfate and the solvent removed *in vacuo* at 40°. When the residual oil (3.22 g.) was allowed to stand in the cold with ligroin (b. p. 30°), 1.57 g. of crystalline product was obtained. Several washes with cold ether and recrystallization from ether–ligroin gave 641 mg. of 7-bromo-Δ^5-androstene-3β,17β-diol 3-acetate-17-benzoate (IIb), m. p. 131–132°; $[\alpha]^{26}D$ −193.5 ± 2° (8.99 mg. in 2.00 ml. of chloroform). From the mother liquor was obtained an additional 338 mg. of the crystalline 7-bromo derivative, m. p. 129–131°, to give a total yield of 979 mg. (40.5%).

Anal. Calcd. for $C_{28}H_{35}O_4Br$: C, 65.23; H, 6.84. Found: C, 65.27; H, 6.92.

7-*d*-Δ^5-Androstene-3β,17β-diol 3-Acetate-17-benzoate (IIIb).—7-Bromo-Δ^5-androstene-3β,17β-diol-3-acetate-17-benzoate (IIb) (543 mg.) in 75 cc. of ethyl acetate was reduced with "deuterized" Raney nickel (prepared by four equilibrations with deuterium oxide, 99.5%) and deuterium (95%) at room temperature and atmospheric pressure. The reduction mixture was worked up as described above to give 459 mg. of bromine-free oil which was dissolved in ligroin and chromatographed on acid-washed alumina. From the benzene–ligroin (9:1, 4:1 and 1:1) eluates 311 mg. of crystalline material was obtained. Recrystallization from ether yielded a mixture of plates and needles. These two types of crystals could be separated by fractional crystallization from hot acetone; the needles (30 mg., m. p. 303–308°) being less soluble in this solvent. The more soluble plates were then recrystallized several times from ligroin (b. p. 90°) to give 189 mg. (41%) of 7-*d*-Δ^5-androstene-3β,17β-diol-3-acetate-17-benzoate (IIIb), m. p. 174–178°; $[\alpha]^{28}D$ −4.8 ± 3° (8.20 mg. in 2.00 ml. of chloroform). When mixed with an authentic sample of Ib, m. p. 177–178°, $[\alpha]^{28}D$ −3.9 ± 2°, there was no depression in melting point. The acetate benzoate (IIIb) contained 1.18 atom per cent. excess deuterium corresponding to 0.43 atom deuterium per molecule. 7-*d*-Δ^5-Androstene-3β,17β-diol (m.p. 180–181°) obtained by the hydrolysis of the above acetate benzoate contained 1.35 atom per cent. excess deuterium corresponding to 0.41 atom deuterium per molecule.

Upon saponification, the mother liquors of the acetate benzoate (IIIb) yielded an additional 24 mg. of the deuterated diol, m. p. 178–179°.

7-Ketocholesterol Acetate Ethylene Mercaptol (VIIIa).—To remove traces of water a small amount of benzene was distilled from a mixture of 10.0 g. of 7-ketocholesterol acetate[20] in 100 cc. of benzene and 20 g. of anhydrous sodium sulfate. To the cooled mixture was added 8 cc. of ethanedithiol and 10 g. of freshly fused zinc chloride. After the reaction mixture was allowed to stand for four days at room temperature, ether was added. The ether extract was washed repeatedly with water, 5% sodium hydroxide solution and water and dried over sodium sulfate. Evaporation of the solvent yielded 14.64 g. of crude mercaptol. Recrystallization from ligroin (b. p. 60°) gave 8.41 g. of rosettes of needles of the ethylene mercaptol of 7-ketocholesterol acetate (VIIIa), m. p. 183–186°. The mother liquor gave a further crop (655 mg.) of mercaptol, m. p. 180–183°. The analytical sample was recrystallized from acetone, m. p. 189.5–190°; $[\alpha]^{26}D$ −81.4 ± 3° (6.88 mg. in 2.00 ml. of chloroform); $\epsilon_{222\,m\mu}$ = 6,430 (95% ethanol).

Anal. Calcd. for $C_{31}H_{50}O_2S_2$: C, 71.77; H, 9.64. Found: C, 71.66; H, 9.33.

The acetate (VIIIa) was saponified by heating for twenty minutes in methanolic sodium hydroxide solution and the product obtained melted at 186–188° (needles from benzene:methanol); a mixture with VIIIa melted at 154–165°. The 3β-hydroxymercaptol was benzoylated at room temperature with benzoyl chloride in pyridine and the product was purified by chromatography on alumina and by recrystallization from benzene:methanol. The benzoate melted at 239–240°. This **7-keto-cholesterol benzoate ethylene mercaptol (VIIIc)** was identical with that prepared directly from 7-ketocholesterol benzoate (m. p. 160°; $[\alpha]^{28}D$ −54.6° (chloroform)) and ethanedithiol in the presence of zinc chloride. The product melted at 241–242.5°; $[\alpha]^{28}D$ −41.2 ± 3° (6.33 mg. in 2.00 ml. of chloroform); $\epsilon_{230\,m\mu}$ = 19,400 (95% ethanol).

Anal. Calcd. for $C_{36}H_{52}O_2S_2$: C, 74.43; H, 9.02. Found: C, 74.54; H, 9.19.

(21) We are grateful to Dr. C. R. Scholz, Ciba Pharmaceutical Co., Summit, New Jersey, for generously supplying us with this material.

7,7-d_2-Cholesterol (IXa).—A solution of 500 mg. of the ethylene mercaptol of 7-ketocholesterol acetate (VIIIa) in 75 cc. of ether was stirred with 5 cc. of "deuterized" Raney nickel for seven hours and then allowed to stand at room temperature for sixteen hours. The catalyst was removed by filtration and washed several times with ethyl acetate. The ether solution and ethyl acetate washings were combined and the solvent evaporated to give 408 mg. of oil. The oil was hydrolyzed by heating with 4 cc. of 5% methanolic potassium hydroxide, 25 cc. of methanol and 1 cc. of benzene for 30 minutes. The crystalline saponified product was purified by chromatography on alumina and three main fractions were obtained. Fraction I, eluted with ligroin, was an oil, 31 mg., which probably was the hydrocarbon produced by hydrogenolysis of the 3-acetoxy group. Fraction II, eluted with benzene: ligroin (1:1), was crystalline cholesterol weighing 171 mg. and melting at 138–142°. Recrystallization from acetone gave a sample melting at 146.5–148.5°; $[\alpha]^{25}D$ −38.1 ± 3° (6.30 mg. in 2.00 ml. of chloroform). Deuterium content of this sample of cholesterol was 2.09 atom per cent. excess deuterium corresponding to 0.96 atom deuterium per molecule. Fraction III, eluted with benzene and ether, proved to be 108 mg. of the hydroxy mercaptol (VIIId). The conditions of desulfurization determined the relative amounts of material in each fraction. Thus, when the mercaptol (1.72 g.) was heated at 100° for six hours with Raney nickel, 632 mg. of fraction I, 462 mg. in fraction II and only 55 mg. in fraction III was obtained. Recrystallization of fraction I from acetone gave a compound melting at 75–77°; $[\alpha]^{26}D$ +21.1 ± 2° (chloroform); the mixed m. p. with cholestane (m. p. 79–80°) was 70–78°. The infrared spectral analysis showed the absence of hydroxyl and acetoxyl groups and the spectrum was similar to that of cholestane. The reported rotation of cholestane is +24.5[22] and of coprostane (m. p. 69–70°) is +25.6.[22] A positive test was obtained with tetranitromethane indicating the presence of an unsaturated hydrocarbon, probably Δ^5-cholestene (reported m. p. 89–91°; $[\alpha]D$ −56.3°).[23]

The yield of hydrocarbon fraction I could be decreased by lowering the temperature, but when this was done, the amount of recovered thioacetal was large. By carrying out the reaction at room temperature for long periods of time,[24] the best yield of desulfurized product (fraction II) was obtained.

3β-Acetoxy-17β-benzoxy-Δ^5-androsten-7-one (IV) from IIb.—Six-tenths gram of 7-bromo-Δ^5-androstene-3β,17β-diol-3-acetate-17-benzoate (IIb) was dissolved in 60 cc. of benzene–ligroin (1:3) and poured through a column of acid-washed alumina. Elutions with benzene–ligroin (1:1), benzene, ether–benzene (1:1), ether and methanol:ether (1:99) gave 11 mg. (A), 33 mg. (B), 329 mg. (C), 34 mg. (D) and 129 mg. (E) of oil, respectively. A portion (225 mg.) of fraction C was dissolved in 2 cc. of glacial acetic acid and oxidized with 3.3 cc. of 2% chromic acid solution. After three hours the excess oxidant was destroyed with methanol and the mixture was taken to dryness *in vacuo*. The residue was extracted twice with ether and the neutral fraction obtained from this extract was semi-crystalline and weighed 215 mg. Fractions B and D were each oxidized in a similar fashion. The oxidation products from the three runs were combined (247 mg.) and were recrystallized from acetone–ether to give 161 mg. of 3β-acetoxy-17β-benzoxy-Δ^5-androsten-7-one (IV), m. p. 203–204°. An additional 35 mg., m. p. 198.5–199°, was obtained from the mother liquor. The analytical sample melted at 205.5–206°; $[\alpha]^{29}D$ −56.0 ± 2° (11.8 mg. in 2.00 ml. of chloroform).

Anal. Calcd. for $C_{28}H_{34}O_5$: C, 74.63; H, 7.61. Found: C, 74.71; H, 7.70.

3β-Acetoxy-17β-benzoxy-Δ^5-androsten-7-one (IV) from Ib.—Following the procedure of Butenandt and co-workers[25] 562 mg. of Δ^5-androstene-3β,17β-diol 3-acetate-17-benzoate in 10 cc. of glacial acetic acid was oxidized at 55° with 450 mg. of chromic acid in 3 cc. of 50% acetic acid solution. The chromic acid solution was added in three portions, 1.3 cc. at the start, 0.5 cc. after fifteen minutes and 1.2 cc. an hour later. The oxidation mixture was kept at 55° for three hours, the excess chromic acid was then decomposed with methanol and the solvent removed *in vacuo*. Water was added to the residue and the mixture then extracted with ether. The ether solution was washed with sodium carbonate solution and water, dried and the solvent evaporated leaving 230 mg. of semi-crystalline residue. After recrystallization from acetone–ether, 118 mg. of 3β-acetoxy-17β-benzoxy-Δ^5-androsten-7-one (IV), m. p. 201–203.5° was obtained. The admixture with the 7-keto derivative obtained above melted at 202–205°.

3β-Acetoxy-17β-benzoxy-Δ^5-androsten-7-one Ethylene Mercaptol (VIIIb).—This mercaptol was prepared from 323 mg. of 3β-acetoxy-17β-benzoxy-Δ^5-androsten-7-one (VIIb) by the procedure described above. The product was purified by chromatography on acid-washed alumina; 277 mg. of the ethylene mercaptol (VIIIb) was obtained from the benzene–ligroin (1:3–1:1) eluates. Recrystallization from acetone gave white needles melting at 215–218.5°. The analytical sample melted at 216–219°; $[\alpha]^{29}D$ −52.3 ± 2° (10.70 mg. in 2.00 ml. of chloroform); $\epsilon_{228\ m\mu}$ = 20,800 (95% ethanol).

Anal. Calcd. for $C_{30}H_{38}O_4S_2$: C, 68.40; H, 7.27. Found: C, 68.28; H, 7.08.

7,7-d_2-Δ^5-Androstene-3β,17β-diol-3-acetate-17-benzoate (IXb) from VIIIb.—A solution of 625 mg. of 3β-acetoxy-17β-benzoxy-Δ^5-androsten-7-one ethylene mercaptol (VIIIb) in 100 cc. of anhydrous ether was stirred with 10 cc. of "deuterized" Raney nickel for ten hours and then allowed to stand for an additional twelve hours. The catalyst was removed by filtration and washed with ethyl acetate. The ether solution and ethyl acetate washings were combined and the solvent removed to give 466 mg. of crystalline residue. Upon chromatographic analysis on acid-washed alumina, 26 mg. of crystalline material was obtained in fraction I (ligroin) which upon recrystallization from ether–methanol gave a product melting at 125–140°. This was not further investigated. From fraction II (benzene–ligroin (1:4) and (1:3)) 158 mg. of 7,7-d_2-Δ^5-androstene-3β,17β-diol 3-acetate-17-benzoate (IXb), m. p. 168–174°, was obtained. Recrystallization from acetone gave heavy plates, m. p. 178–179°; $[\alpha]^{25}D$ −4.9 ± 2° (10.6 mg. in 2.00 ml. of chloroform). Deuterium content of this sample was 4.00 atom per cent. excess deuterium, corresponding to 0.96 atom deuterium per molecule. 7,7-d_2-Δ^5-Androstene-3β,17β-diol obtained from the hydrolysis of the above sample contained 4.15 atom per cent. excess deuterium, corresponding to 0.65 atom deuterium per molecule. This decrease in isotope concentration indicates that some of the hydrogen on the ester groups had exchanged. Fraction III (benzene:ligroin (1:1), benzene and ether) weighed 216 mg. and was not homogeneous. It was therefore repurified by chromatographic analysis to yield an additional 14 mg. of IXb and 120 mg. of the thioacetal VIIIb, m. p. 215–221°.

Cholestan-3-one Ethylene Mercaptol.—Cholestan-3-one (300 mg.) was condensed with ethanedithiol (0.4 cc.) in the manner described above for 7-ketocholesterol acetate. The semi-crystalline reaction product weighed 352 mg. and was recrystallized twice from ligroin (b. p. 60°) to give an analytical sample, m. p. 142–143°; $[\alpha]^{32}D$ +29.7 ± 1° (8.76 mg. in 2.00 ml. of chloroform); $\epsilon_{240\ m\mu}$ = 333 (95% ethanol).

Anal. Calcd. for $C_{25}H_{30}S_2$: C, 75.26; H, 10.89. Found: C, 75.39; H, 10.88.

Summary

Two methods for the introduction of stably bound deuterium into the steroid nucleus have been investigated.

(22) Mauthner, *Monatsh.*, **30**, 635 (1909).

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

(24) McIntosh, Meinzer and Levin, THIS JOURNAL, **70**, 2955 (1948).

(25) Butenandt, Hausmann and Paland, *Ber.*, **71**, 1316 (1938).

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.

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[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.

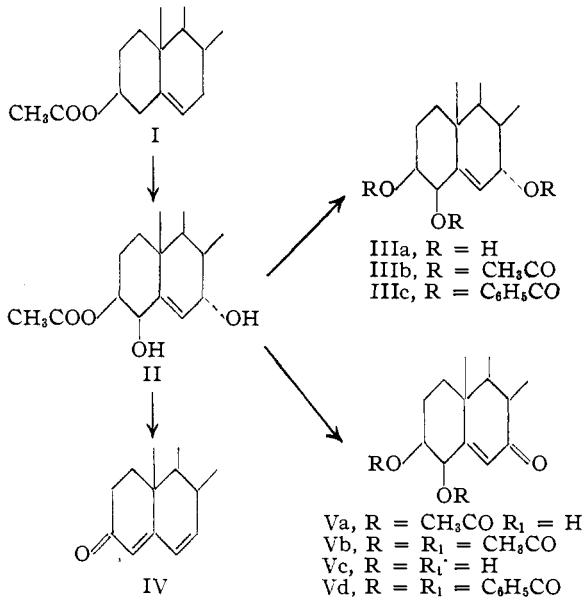

Fig. 1.

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°	

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

(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.