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THE PREPARATION OF SOME STEROIDS CONTAINING DEUTERIUM[1]

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ABSTRACT

The preparation of several deuterated steroids is described, viz.,

(i) the trideuteroacetates of the following alcohols: $\Delta^{5,7,9}$-estratrienol-17β; $\Delta^{1,3,5:10}$-estratrienol-3-one-17 (*estrone*); androstanol-3α; androstanol-3β; androstanol-17β; pregnanol-20α; Δ^{5}-pregnenol-3β-one-20; Δ^{5}-pregnenol-3β-one-20-d_4-17,21; Δ^{5}-cholestenol-3β; $\Delta^{6,8}$-cholestadienol-3β; ergostanol-3β; Δ^{14}-ergostenol-3β; Δ^{22}-5-isoergostenol-3α.

(ii) Δ^{5}-pregnenol-3β-one-20-d_3-21 acetate; Δ^{5}-pregnenol-3β-one-20-d_4-17,21.

(iii) androstanone-3-d_4-2,4; cholestanone-3-d_4-2,4; $\Delta^{8:14}$-ergostenone-3-d_4-2,4; cholestanone-7-d_2-6; androstanone-17-d_2-16.

From difficulties encountered in the preparation of cholestanone-7-d_2-6, it is inferred that 7-ketones enolize less readily than 3-,17-, or 20-ketones.

INTRODUCTION

Although a number of steroids containing deuterium have been described in the literature, previous investigators have sought mainly to introduce deuterium atoms into tightly bound positions, to serve as labels in studies of steroid metabolism. The methods most commonly employed have involved the exchange of steroids with deuterium oxide and acetic acid in the presence of platinum catalysts (1, 2, 4), or the catalyzed addition of deuterium to unsaturated steroids (10, 12, 13). Recently, introduction of deuterium has also been effected by treatment of steroid bromides or mercaptols with "deuterized" Raney nickel (5).

This paper is concerned with the preparation of several steroids in which deuterium atoms are introduced at relatively labile positions, viz.,

(i) as CD_3 groups in the acetoxy radicals of steroid alcohols acetylated with acetic anhydride-d_6.

(ii) at C_{17} and C_{21} in the side chain of Δ^{5}-pregnenol-3β-one-20 (Δ^{5}-*pregnenolone*).

(iii) as CD_2 groups adjacent to the carbonyl groups of 3-, 7-, and 17-ketosteroids.

These compounds have been prepared for the study of their infrared absorption spectra (8), and a comparison of the positions of the infrared absorption bands in the normal and deuterized steroids has aided in the assignment of the bands

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between 1350 and 1500 cm.$^{-1}$ to the vibrations of specific methyl and methylene groups in the steroid molecule (7).

I. *Steroid Acetates*

Acetic anhydride-d_6 was synthesized from acetyl-d_3 chloride (3) and sodium acetate-d_3, both the acid chloride and the sodium salt themselves being prepared from acetic-d_3 acid (3, 6).

Thirteen steroid alcohols were acetylated with heavy acetic anhydride (Table I). In an initial investigation cyclohexyl acetate-d_3 was also prepared by direct esterification with acetic-d_3 acid in acidic solution.

II. *Steroids Containing the $-CO-CD_3$ Side Chain*

The method employed for the introduction of the CD_3 group into the side chain of Δ^5-pregnenolone was similar to that used by MacPhillamy and Scholz (11) for the preparation of 21-C^{14}-progesterone. Δ^5-3β-acetoxyetiocholenic acid chloride (I) was treated with dimethylcadmium-d_6 to yield Δ^5-pregnenolone-d_3-21 acetate (II). The organocadmium compound was prepared *in situ* from the Grignard derivative of methyl-d_3 bromide.

When Δ^5-pregnenolone-d_3-21 acetate was hydrolyzed in the normal manner with sodium hydroxide in aqueous ethanol, the product isolated contained no excess deuterium and the infrared absorption spectrum was identical with that of normal Δ^5-pregnenolone (VII). It was therefore evident that the deuterium atoms in the C_{21}-d_3 group were lost during the hydrolysis of the acetate group, presumably as a result of enolization of the 20-ketone in alkaline solution.

The deuterated C_{21} methyl group was protected by carrying out the hydrolysis of the acetate with sodium carbonate in deuterium oxide and methanol-*d*. By this procedure some deuterium was introduced at C_{17} and some also into the hydroxyl group (III); the latter was readily displaced by exchange with methanol in neutral solution to yield Δ^5-pregnenolone-d_4-17,21 (IV). Both the light and heavy acetates of (IV) were also prepared (V, VI).

Since enolization of the 20-keto group occurred during the alkaline hydrolysis of the acetate group, there existed a possibility that the product might contain appreciable quantities of Δ^5-17-iso-pregnenolone-d_4-17,21. This was largely ruled out by the fact that a sample of normal Δ^5-pregnenolone prepared from I by the same procedure gave the correct optical rotation. The molecular rotations of Δ^5-pregnenolone and Δ^5-17-isopregnenolone are $+89°$ and $-433°$ respectively (in acetone solution) so that quite small traces of the 17-stereoisomer would have been detected by this means.

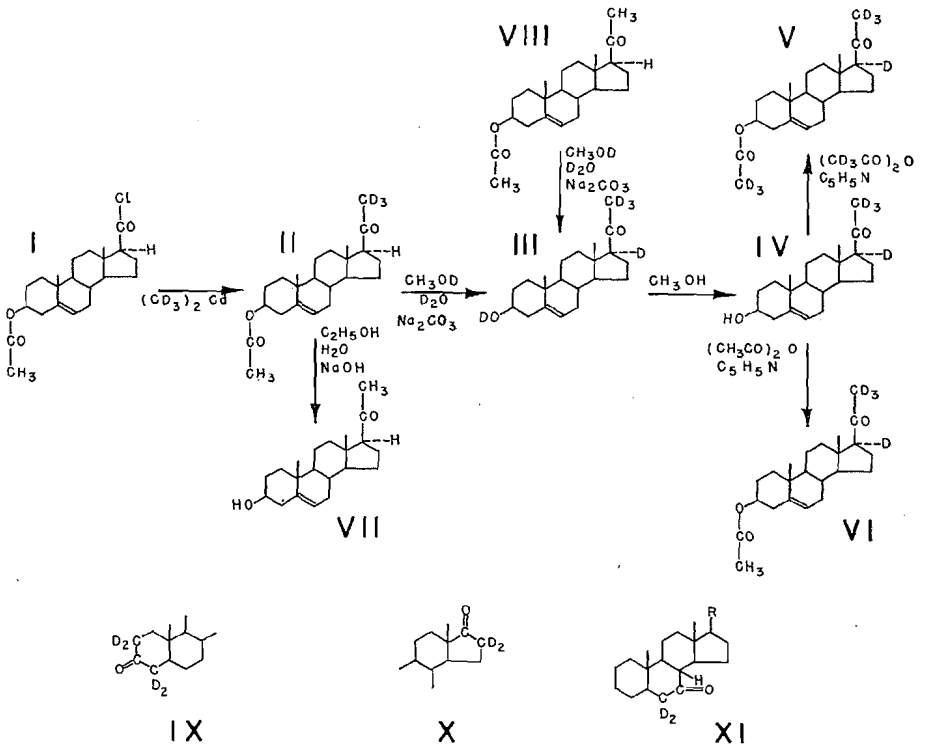
Δ^5-Pregnenolone-d_4-17,21 was also prepared directly by hydrolysis of Δ^5-pregnenolone acetate (VIII) with sodium carbonate in deuterium oxide and methanol-*d*. The resultant deuterated Δ^5-pregnenolone, after treatment with methanol and purification, gave an infrared spectrum identical with that of (IV), and contained 3.4 atoms of deuterium per molecule.

III. *Ketosteroids Deuterated in the α-Methylene Groups*

The 3-ketosteroids listed in Table II, on treatment with sodium carbonate in deuterium oxide and methanol-*d*, underwent deuterium exchange to the extent

of 3.7–3.8 atoms of deuterium per molecule, from which it is inferred that the methylene at both C_2 and C_4 participate in the enolization to yield IX as the principal product. Under the same conditions androstanone-17 exchanged to the extent of 2.12 atoms of deuterium per molecule. Since the 17-ketone can enolize in one way only, the product must be principally the C_{16}-d_2 derivative (X).

When cholestanone-7 was treated in a similar manner to the 3-, 17-, and 20-ketosteroids, the product contained only 0.59 atom of deuterium per molecule, and even under more forcing conditions of enolization, using sodium deuteroxide instead of sodium carbonate, the deuterium content could not be increased above 1.81 atoms per molecule.



Since 7-ketosteroids contain three potentially enolizable hydrogen atoms, two at C_6 and one at C_8, the structure of this product is in doubt. It is assumed tentatively that the major component has the C_6-d_2 structure (XI), but some material containing deuterium at C_8 may also be present. The infrared absorption spectrum does not serve to differentiate these; the disappearance of an absorption band at 1433 cm.$^{-1}$ which is observed to occur on deuteration of cholestanone-7 (8) requires only that *one* of the hydrogen atoms at C_6 be exchanged.

EXPERIMENTAL*

I. *Steroid Acetates*

Acetic Anhydride-d_6

Acetic-d_3 acid, prepared by the decarboxylation of malonic acid exchanged with deuterium oxide, was neutralized with aqueous sodium carbonate solution. The solution was evaporated to dryness and the resultant trihydrate decomposed by heating at 160° C. under vacuum. One equivalent of acetyl-d_3 chloride was added dropwise on to the dry salt and the anhydride distilled off. B.p. 137.0–138.2° C.; n_D^{20} 1.3881.

Cyclohexyl Acetate-d_3

A mixture of freshly distilled cyclohexanol (2.0 ml.), acetic-d_3 acid (2.5 ml.), and concentrated sulphuric acid (0.13 ml.) was refluxed for one hour. The cooled reaction mixture was diluted with water and extracted with ether. The ethereal solution was washed with aqueous sodium hydroxide, water, and dried with anhydrous sodium sulphate. After removal of the ether the residue was fractionally distilled under reduced pressure. B.p. 174.2–174.8° C.; n_D^{20} 1.4413.

Steroid Trideuteroacetates

The steroid alcohols (10–20 mgm.) were dissolved in dry pyridine (0.2 ml.), an equal volume of acetic anhydride-d_6 was added, and the mixture allowed to stand at room temperature overnight. The excess acetic anhydride-d_6 and pyridine were removed by vacuum distillation, and the residual trideuteroacetate purified by crystallization or high vacuum sublimation. The physical constants of the trideuteroacetates prepared in this manner are listed in Table I.

TABLE I
STEROID TRIDEUTEROACETATES

Steroid alcohol	M.p., °C.	
	Trideuteroacetate	Normal acetate
$\Delta^{5,7\ 9}$-Estratrienol-17β	98.0—101.0	—
Estrone	122.4—124.4	125.2—126.2
Androstanol-3α	133.6—134.6	133.2—134.0
Androstanol-3β	85.5— 86.4	85.0— 86.2
Androstanol-17β	75.5— 77.5	77.3— 79.3
Pregnanol-20α	127.3—127.8	—
Δ^5-Pregnenol-3β-one-20	141.3—142.5	142.5—144.7
Δ^5-Pregnenol-3β-one-20-d_4-17,21	140.2—141.9	142.5—144.7
Δ^5-Cholestenol-3β	112.7—114.4*	114.2—114.8
$\Delta^{6,8}$-Cholestadienol-3β	100.0—102.0	—
Ergostanol-3β	143.9—144.6	142.4—143.4
Δ^{14}-Ergostenol-3β	87.0— 88.0	90.0— 91.5
Δ^{22}-5-Isoergostenol-3α	108.9—110.2	106.6—109.4

* *Deuterium content 2.7 atoms per molecule.*

II. *Steroids Containing the $-CO-CD_3$ Side Chain*

Methyl-d_3 Bromide

Dry silver acetate-d_3 (31 gm.) was suspended in carbon tetrachloride (125 ml.)

* *All melting points are corrected.*

in a three-necked 0.5 liter flask provided with a dropping funnel, a gas inlet tube, and an efficient vertical water-cooled condenser. The upper end of the condenser led into two gas wash-bottles containing 40% potassium hydroxide solution, followed by two traps cooled in dry ice – acetone to receive the crude methyl-d_3 bromide.

The reaction mixture was cooled in iced water and stirred magnetically while bromine (13 ml.) was added dropwise at a rate sufficient to maintain a steady evolution of carbon dioxide without inducing an excessive temperature rise. After the bromine was added, the flask was warmed slowly and refluxed until the evolution of gas diminished. The mixture was then allowed to cool and a stream of nitrogen passed through the system for 30 min. The material collected in the traps was dried with Drierite and submitted to repeated fractional distillation. Some 2.4 ml. of deuterated methyl bromide were obtained after four distillations, corresponding to 25% of the theoretical yield.* The deuterium content, as determined by mass spectrometry, was 2.7 deuterium atoms per molecule.

Δ^5-Pregnenolone-d_3-21 Acetate (II)

The Grignard derivative of methyl-d_3 bromide was prepared from magnesium turnings (0.275 gm.) and methyl-d_3 bromide (0.65 ml.) in dry ether (20 ml.). Cadmium bromide (3.0 gm.) was added and the reaction mixture refluxed for two hours with magnetic stirring. A solution of Δ^5-3β-acetoxyetiocholenic acid chloride (I) (2.14 gm.) in benzene (20 ml.) was added dropwise, followed by 5 ml. of benzene. The reaction mixture was refluxed for two hours, and then cooled to room temperature. The product was decomposed with water (8 ml.) and 10% hydrochloric acid (12 ml.), stirred for 30 min., and allowed to stand overnight.

The product was extracted with ether and the ethereal solution, after washing successively with 5% sodium bisulphite, sodium hydroxide, and water, was dried with anhydrous sodium sulphate. On removal of the solvent there remained 1.25 gm. of crude Δ^5-pregnenolone-d_3-21 acetate (II). After two recrystallizations from methanol and a third from methanol–acetone–water the product melted at 138.2–140.2° C. and contained 2.67 deuterium atoms per molecule. The infrared absorption spectrum contained two maxima in the C = O stretching region at 1732 and 1702 cm.$^{-1}$ respectively in CCl_4 solution, indicative of the presence of the acetate and the 20-ketone group respectively (9).

The crude ester (0.77 gm.) was treated with 40 ml. of 0.1 *N* sodium hydroxide in 65% aqueous ethanol for 30 min. On working up the reaction product normal Δ^5-pregnenolone (VII) was isolated (m.p. 181.1–182.6° C.) with an infrared spectrum indistinguishable from that of the normal light compound, and exhibiting no deuterium enrichment.

Δ^5-Pregnenolone-d_4-17,21 (IV)

Crude Δ^5-pregnenolone-d_3-21 acetate (95 mgm.) was dissolved in a solution of anhydrous sodium carbonate (50 mgm.) in methanol-*d* (2.0 ml.) and deu-

* *The yields in this reaction were variable and in some preliminary runs with light silver acetate yields exceeding 50% were obtained. An improved method for the preparation of methyl-d_3 bromide from silver acetate-d_3 and bromine in a sealed vessel has subsequently been developed and will be described elsewhere.*

terium oxide (0.2 ml.). The solution was warmed on a water bath and a precipitate separated out. The precipitate was redissolved by the addition of 2.0 ml. of methanol-*d* and the solution refluxed for two hours. The alcohol was distilled off, the residue acidified with 5% aqueous hydrochloric acid, and the resultant suspension extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulphate, and the solvent removed. The product (III) assayed for 4.62 atoms of deuterium per molecule. It was treated several times with methanol and finally recrystallized from ether at −78° C. (m.p. 180.0–182.4° C.). The Δ^5-pregnenolone-d_4-17,21 (IV) so obtained assayed for 3.66 atoms of deuterium per molecule. The loss of 0.96 atom of deuterium per molecule on treatment of III with methanol in neutral solution was attributed to exchange of deuterium out of the hydroxyl group, and indicated the stability of the deuterium at C_{17} and C_{21} in neutral solution.

Δ^5-Pregnenolone-d_4-17,21 Acetate-d_3 (V)

This was prepared from (IV) by acetylation with acetic anhydride-d_6 as described above (Table I).

Δ^5-Pregnenolone-d_4-17,21 (IV) from Δ^5-Pregnenolone Acetate (VIII)

Δ^5-Pregnenolone acetate (6 mgm.) was dissolved in a solution of anhydrous sodium carbonate in methanol-*d* and deuterium oxide, the solution was refluxed overnight, and the product worked up as described above for (IV). The crude product was purified by high vacuum sublimation to yield 4.5 mgm. of material (m.p. 180.4–181.4° C.) which gave an infrared absorption spectrum identical with (IV) and contained 3.36 deuterium atoms per molecule.

Deuterium Content of Above Compounds

Since the deuterated methyl bromide, Δ^5-pregnenolone-d_3-21 acetate, and Δ^5-cholestenol-3β-acetate-d_3 all assayed for 2.7 atoms of deuterium per methyl group it seems probable that this corresponds to the deuterium content of the acetic acid used as starting material for all three preparations, and that no loss of deuterium by exchange occurred during the reactions described. This interpretation is supported by the fact that methane prepared from the same batch of acetic-d_3 acid by pyrolysis of the sodium salt with soda lime also assayed for 2.7 atoms of deuterium per molecule. Technical difficulties in sample preparation and dilution precluded an accurate evaluation of the deuterium content of the acetic-d_3 acid or acetic anhydride-d_6 by mass spectrometry.

III. *Ketosteroids Deuterated in the α-Methylene Group*

The ketosteroid (25 mgm.) was dissolved in methanol-*d* (4 ml.) and deuterium oxide (0.5 ml.), and 5 mgm. of anhydrous sodium carbonate were added. The reaction mixture was refluxed for 10 min., and evaporated to dryness. The residue was taken up in 4 ml. of methanol-*d* and 0.5 ml. of deuterium oxide, the solution was evaporated to dryness, refluxed, and the cycle repeated. The product was then extracted with anhydrous ether and the residue from the ethereal extract purified by high vacuum sublimation. The melting points and deuterium assays of the ketosteroids treated in this manner are listed in Table II.

In the case of cholestanone-7 it was necessary to replace the sodium carbonate by sodium deuteroxide to effect appreciable exchange.

TABLE II
DEUTERATED KETOSTEROIDS

Ketosteroid	M.p., °C.	Deuterium atoms per molecule
Androstanone-3	98.7— 99.6	3.66
Cholestanone-3	129.1—129.5	3.69
$\Delta^{8,14}$-Ergostenone-3	122.5—125.5	3.80
Cholestanone-7	109.0—112.5	1.81
Androstanone-17	121.0—121.8	2.12

CONCLUDING REMARKS

Since the infrared absorption spectra of these compounds form the subject of a separate communication (8) they will not be discussed in detail here.

The suggestion from the ketone exchange reactions that the 7-ketone is less readily enolized then the 3-, 17-, or 20-ketosteroids might have useful implications in synthetic work. It also suggests that a study of the effects of hydrogen ion concentration, and other reaction conditions, on the rate and extent of deuterium exchange could provide rather precise information about the relative reactivities of carbonyl groups. Infrared spectrometry could be employed effectively to follow the progress of such exchange reactions, since enolizable methylene and methyl groups give rise to characteristic infrared absorption bands between 1350 and 1440 cm.$^{-1}$ (7). These bands all disappear on the introduction of a deuterium atom into the enolizable methylene group.

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