[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Reduction of Unsaturated Steroids with Deuterium: Distribution of Isotope and Mechanism[1]

BY DAVID K. FUKUSHIMA AND T. F. GALLAGHER

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It is shown that various unsaturated steroids are reduced with deuterium in acetic acid-*d* and a platinum catalyst with the incorporation of significantly more than two gram atoms of isotope per mole of compound reduced. The location of the isotope in cholestanol obtained from cholesterol under these conditions was investigated. It has been concluded that 0.9, 1.25 and 0.40 gram atoms of deuterium per mole were attached to C-5, C-6 and C-7, respectively, and a mechanism to account for this distribution has been proposed. The incorporation of almost exactly 2 atoms of isotope when methyl 3α-acetoxy-Δ^{11}-cholenate was reduced under similar conditions is explicable in terms of this mechanism.

It might be expected that catalytic reduction with deuterium of an isolated aliphatic double bond would result in the introduction of but 2 atoms of the isotope. Indeed, in view of the isotopic fractionation between protium and deuterium,[2] slightly less, rather than more isotope would be incorporated unless great care was taken to exclude protium from the solvent and other components of the hydrogenation system. However, in a series of steroids, the platinum catalyzed reduction of an isolated double bond with deuterium gas in a medium of acetic acid-*d*, with a single exception, was accomplished with the incorporation of significantly more than 2 D (gram atoms of deuterium per mole of compound). Thus cholesterol acetate, hydrogenated under these conditions and saponified, yielded cholestane-3β-ol with 2.55 D. Methyl 3α-acetoxy-$\Delta^{9(11)}$-cholenate was similarly reduced to methyl 3α-acetoxycholanate with the incorporation of 2.21 D. The presence of the secondary alcohol group at C-3 was shown to be without influence on this reaction when Δ^5-cholestene, under analogous conditions, was reduced to a product containing 2.67 D. The reduction of a tertiary carbon was similarly not the controlling factor since Δ^2-cholestene yielded cholestane with 2.42 D. The long side chain of either sterols or bile acids was without influence upon reduction as evidenced by the fact that dehydroisoandrosterone acetate after complete reduction in acetic acid-*d* followed by reoxidation and equilibration afforded isoandrosterone containing 2.29 D together with 3β-hydroxyetiocholane-17-one containing 2.31 D. The same treatment of 3β-acetoxy-Δ^5-pregnene-20-one afforded 3β-hydroxyallopregnane-20-one with 2.28 D.

The catalytic reduction of methyl 3α-acetoxy-Δ^{11}-cholenate to methyl 3α-acetoxycholanate under similar conditions but with the rigid exclusion of contamination by protium had been shown in previous studies[2] to proceed with the incorporation of the theoretical amount of 2 gram atoms of deuterium per mole of compound. Since the catalytic reductions reported in this paper were conducted under less rigorous conditions in that the deuterium gas was stored over ordinary water with a Dry Ice trap interposed between the gas and the solution containing the substrate and catalyst, the hydrogenation of methyl 3α-acetoxy-Δ^{11}-cholenate was repeated with these conditions and the product obtained had 1.77 D. It is thus apparent that the values reported for the several reduction products are all significantly lower than would have been obtained had the most rigid precautions been taken to exclude contamination with protium; the high incorporation of isotope is therefore actually less than theoretically attainable. This shortcoming in the quantitative aspects of the study has no bearing, however, upon the general validity of the phenomenon observed.

The mechanism by which hydrogenation occurred simultaneously with the incorporation of more than 2D of reduced compound is of particular interest in connection with the generalized theory of hydrogen transfer in catalysis. In view of the numerous steric factors operative, as well as the possibility of well controlled degradation, the steroids are a particularly favorable class of compounds for the study of this problem. On first inspection of the results reported it might be assumed that a reaction had occurred similar to the exchange operative when ethylene was reduced with deuterium in the gas phase.[3] If this were the case, it would be expected that the 2.55 D incorporated into cholesterol upon catalytic reduction under the specified conditions would be distributed among the three hydrogen atoms at C-5 and C-6 of the steroid nucleus. Oxidation of cholestanol-*d* followed by equilibration of the ketone with alkali in order to exchange any enolizable isotope with the medium demonstrated that there was no significant change in the isotopic concentration. There was therefore no isotope at C-4 and this was considered to be in accord with the hypothesis advanced. This result, likewise, appeared to exclude the possibility of double bond migration in the course of catalytic reduction since this mechanism might be expected to lead to the incorporation of surplus isotope at both C-4 and C-7 as well as at C-5 and C-6. Although these results seemed to support the exchange hypothesis it was desirable to determine the precise location of the additional isotope introduced in the course of catalytic hydrogenation in order to establish conclusively the details of the reaction.

The isotopic cholestane-3β-ol was converted to Δ^4-cholestene-3-one and equilibrated with base to

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(2) M. L. Eidinoff, J. E. Knoll, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, 74, 5280 (1952).

(3) A. Farkas, L. Farkas and E. K. Rideal, *Proc. Roy. Soc. (London)*, 146A, 630 (1934).

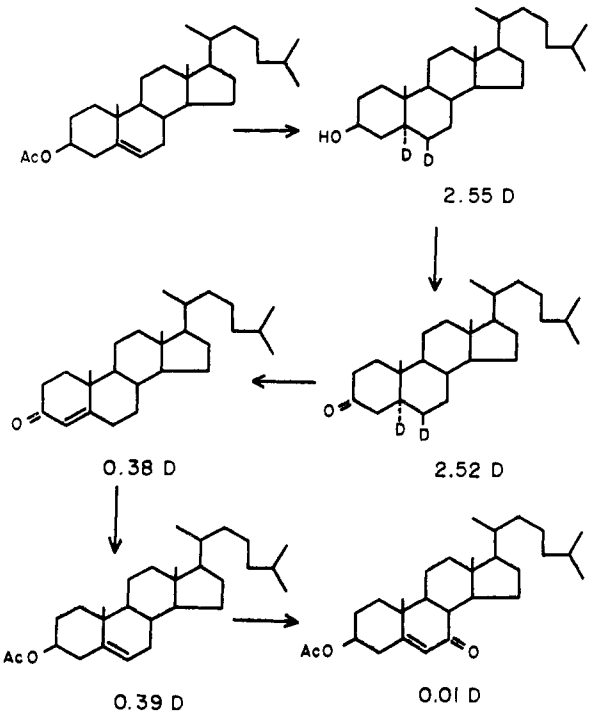

Fig. 1.

remove any isotope at C-2, C-4 and C-6. These reactions were accomplished with the loss of 2.15 D *but with the retention of 16% of the total isotope incorporated* (Fig. 1). It was immediately apparent that a more complicated process than hydrogen exchange on the single ethylenic carbon (C-6) capable of reaction was involved in the introduction of more than the calculated isotope. Since an exchange reaction of the type encountered at elevated temperatures[4,5] was scarcely likely under the experimental conditions of reduction, it seemed reasonable that the additional stable isotope in the equilibrated cholestenone-*d* was at C-7, the carbon atom immediately adjacent to the olefinic bond of the compound reduced. Confirmation of this hypothesis was obtained from the following series of reactions. Cholestenone-*d* was converted to cholesterol by the method of Belleau and Gallagher.[6] Cholesterol acetate was oxidized with *t*-butyl chromate[7] and the product, 7-ketocholesterol acetate, was found to be devoid of isotope. It was thus shown that the 2.55 D introduced into cholesterol acetate by catalytic reduction in acetic acid-*d* was distributed among C-5, C-6 and C-7 and that 0.40 D was certainly at C-7. From considerations that will be dealt with in the discussion it is probable, likewise, that of the remaining isotope, 0.9 D was at C-5 and 1.25 D was at C-6.

Experimental[8]

Cholestane-3β-ol.—A solution of 3.50 g. of cholesterol acetate in 200 ml. of acetic acid-*d* (prepared by hydrolysis of purified acetic anhydride with 99.9% deuterium oxide) was hydrogenated in the presence of 700 mg. of Adams catalyst with 99.5% deuterium gas at room temperature. The deuterium gas was stored over ordinary water and a Dry Ice trap was interposed between the gas reservoir and the reaction vessel; connection between the two was made with "Tygon" tubing. The reduction mixture was isolated and the crude cholestanol acetate was purified by the method of Anderson and Nabenhauer.[9] The acetate was hydrolyzed by heating under reflux for 1 hour with 125 ml. of methanol, 10 ml. of benzene and 75 ml. of 5% methanolic potassium hydroxide. After isolation in the appropriate manner and recrystallization from acetone, 1.63 g. of cholestanol, m.p. 141–142°, was obtained. The product contained 5.31% Ex. (atoms percentage excess deuterium) corresponding to 2.55 D.

Cholestane-3-one.—The product described above was diluted with non-isotopic cholestanol to a concentration of 0.554% Ex. A solution of 15.5 g. of dilute cholestanol was oxidized with 21 g. of sodium dichromate, 16 ml. of acetic acid, 28 ml. of concentrated sulfuric acid and 95 ml. of water.[10] Recrystallization from methanol gave 12.5 g. of cholestane-3-one, m.p. 129.5–130° containing 2.52 D (calculated for the undiluted compound). The mother liquor yielded an additional 0.8 g. of cholestane-3-one, m.p. 129–130°.

One hundred mg. of the deuterium containing cholestane-3-one was equilibrated by heating under reflux for 4 hours with 25 ml. of methanol, 25 ml. of 5% methanolic potassium hydroxide and 4 ml. of water. The equilibrated cholestanone contained 2.48 D (calculated for the undiluted compound).

Δ^4-Cholestene-3-one.—The above described cholestane-3-one was converted to Δ^4-cholestene-3-one by the method of Rosenkranz, *et al.*[11] After chromatography on alumina, 6.5 g. of crude Δ^4-cholestene-3-one was isolated and equilibrated by heating for 5 hours under nitrogen with 300 ml. of methanol, 270 ml. of 5% methanolic potassium hydroxide and 30 ml. of water. The product was again chromatographed and the purest fraction was recrystallized twice from methanol and from acetone to yield Δ^4-cholestene-3-one, m.p. 81–82°, ϵ_{2420} 16,400 (ethanol), 0.38 D (calculated for the undiluted compound). A portion of this product was converted to the hydrazone by treatment with Girard reagent T. The complex with the Girard reagent was selectively dissociated[12] by allowing the neutralized aqueous solution to stand at room temperature followed by ether extraction. The ether extract was discarded. The aqueous solution was acidified to congo red and heated on a steam-bath for 45 minutes. After extraction with ether, the solvent was removed and the residue obtained was sublimed in a high vacuum; after recrystallization from methanol and from acetone Δ^4-cholestenone was obtained, m.p. 82–83°, ϵ_{2420} 16,200 (ethanol), 0.40 D (calculated for the undiluted compound).

The remainder of the Δ^4-cholestene-3-one was combined and recrystallized to give 4.40 g. of Δ^4-cholestene-3-one, m.p. 78–80°, ϵ_{2420} 16,100 (ethanol).

Cholesterol.—The foregoing Δ^4-cholestene-3-one was converted by the method of Belleau and Gallagher,[6] to 2.30 g. of highly purified cholesterol, m.p. 148.5–149.5°. Acetylation with acetic anhydride and pyridine at room temperature afforded the acetate, m.p. 114.5–115°, 0.39 D (calculated for the undiluted compound).

7-Ketocholesterol Acetate.—The above cholesterol acetate was oxidized to the 7-keto derivative by the method of Oppenauer and Oberrauch.[7] Purification of the product followed by recrystallization from methanol and from acetone afforded 7-ketocholesterol acetate, m.p. 158.5–160°, ϵ_{2360} 12,900 (ethanol), 0.01 D within the analytical limits of error, the compound contained the normal abundance of deuterium.

Reduction of Dehydroisoandrosterone Acetate.—A solution of 6.60 g. of dehydroisoandrosterone acetate in 70 ml. of acetic acid-*d* was hydrogenated with 99.5% deuterium gas in the presence of 600 mg. of Adams catalyst. A portion

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(5) D. K. Fukushima and T. F. Gallagher, *ibid.*, **198**, 861 (1952).
(6) B. Belleau and T. F. Gallagher, THIS JOURNAL, **73**, 4458 (1951).
(7) R. V. Oppenauer and H. Oberrauch, *Anales asoc. quim. argentina*, **37**, 246 (1949).
(8) All melting points are corrected.
(9) R. J. Anderson and F. P. Nabenhauer, THIS JOURNAL, **46**, 1957 (1924).
(10) "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 139.
(11) G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, THIS JOURNAL, **72**, 4077 (1950).
(12) T. Reichstein, *Helv. Chim. Acta*, **19**, 1107 (1936).

(2.75 g.) of the reduction product was oxidized with 2.00 g. of N-bromoacetamide, in 25 ml. of *t*-butyl alcohol and 0.5 ml. each of water and pyridine. The crude product was saponified with methanolic potassium hydroxide solution. Recrystallization from acetone gave isoandrosterone-5,6-d_2, m.p. 175.5–176.5°, $[\alpha]^{22}D$ +89.6 ($CHCl_3$), 2.29 D. Chromatography of the mother liquors on alumina afforded 3β-hydroxyetiocholane-17-one which upon recrystallization from ether gave needles, m.p. 154.5–156°, $[\alpha]^{22}D$ +93.7 ($CHCl_3$), 2.31 D.

Reduction of 3β-Acetoxy-Δ⁵-pregnene-20-one.—A solution of 6.86 g. of 3β-acetoxy-Δ⁵-pregnene-20-one in 85 ml. of acetic acid-*d* was hydrogenated with deuterium gas in the presence of Adams catalyst. The crude reduction product was oxidized with 5.52 g. of N-bromoacetamide, 45 ml. of *t*-butyl alcohol, 2 ml. of water and 2 ml. of pyridine. Recrystallization from petroleum ether and methanol gave 3.79 g. of 3β-acetoxyallopregnane-20-one, m.p. 146.5–147°, 2.28 D.

Reduction of Δ⁵-Cholestene.—A solution of 250 mg. of Δ⁵-cholestene in 50 ml. of acetic acid-*d* was hydrogenated with 99.5% deuterium gas in the presence of 50 mg. of Adams catalyst. Recrystallization of the reduction product from acetone gave cholestane, m.p. 80.5–81.5°, $[\alpha]^{25}D$ +25° (chloroform), 2.66 D. The crystalline hydrocarbon was dissolved in benzene and treated with perbenzoic acid for 24 hours. Chromatography and recrystallization from acetone gave cholestane, m.p. 77–78°, 2.67 D.

Reduction of Δ²-Cholestene.—A solution of 100 mg. of Δ²-cholestene in 55 ml. of acetic acid-*d* was hydrogenated with 99.5% deuterium gas in the presence of 30 mg. of Adams catalyst. Recrystallization of the reduction product from acetone gave cholestane, m.p. 80–80.5°, $[\alpha]^{24}D$ +26.2° (chloroform), 2.45 D. After treatment with perbenzoic acid, chromatography and recrystallization from acetone, cholestane, m.p. 78–80°, 2.42 D, was obtained.

Reduction of Methyl 3α-Acetoxy-Δ¹¹-cholenate.—A solution of 125 mg. of methyl 3α-acetoxy-Δ¹¹-cholenate in 30 ml. of acetic acid-*d* was hydrogenated with 99.5% deuterium gas in the presence of 30 mg. of Adams catalyst. Recrystallization from petroleum ether and acetone gave methyl 3α-acetoxycholanate, m.p. 135–136°, 1.82 D. Treatment with perbenzoic acid, chromatography on silica gel and recrystallization from acetone gave methyl 3α-acetoxycholanate, m.p. 135–136°, 1.77 D.

Reduction of Methyl 3α-Acetoxy-Δ⁹⁽¹¹⁾-cholenate.—A solution of 250 mg. of methyl 3α-acetoxy-Δ⁹⁽¹¹⁾-cholenate in 50 ml. of acetic acid-*d* was hydrogenated with 99.5% deuterium gas in the presence of Adams catalyst. Recrystallization from petroleum ether afforded a product, m.p. 134–135° which was unsaturated as judged by test with tetranitromethane. The reduction product was treated with 5 ml. of a benzene solution of perbenzoic acid (30 mg./ml.) for 3 days at room temperature. The reaction mixture was worked up in an appropriate manner and the product was chromatographed on silica gel to yield 132 mg. of methyl 3α-acetoxycholanate. Recrystallization of methyl 3α-acetoxycholanate from acetone gave a product, m.p. 135.5–136°, $[\alpha]^{25}D$ +46.3° (acetone), 2.26 D. The compound was hydrolyzed with 15 ml. of 2% methanolic potassium hydroxide at room temperature for 30 minutes. Recrystallization gave lithocholic acid, m.p. 187–189°. Treatment with diazomethane, chromatography on silica gel and recrystallization from acetone gave methyl lithocholate, m.p. 130–131°, $[\alpha]^{30}D$ +32.7° (acetone), 2.21 D.

Deuterium Analysis.—The analyses for deuterium were made by combustion of the steroid in a stream of oxygen and passage of the water vapor through a zinc-filled furnace at about 400° in a good vacuum. The ratio of hydrogen to deuterium was determined in the mass spectrometer.

Discussion

The simultaneous exchange of deuterium for protium during catalytic hydrogenation was first observed with ethylene and nickel in 1934.[3] Subsequently other studies of catalytic hydrogenation and exchange with deuterium gas and hydrocarbons in the vapor phase have resulted in a number of proposed mechanisms for the reaction.[13] In order to explain the distribution of deuterium in the platinum catalyzed hydrogenation of cholesterol and other steroids in acetic acid-*d* (CH_3COOD), a modified form of the "associative" mechanism[14,15] seems best applicable. The activation of the olefin linkage is depicted as a π-bond between the electrons of the double bond and the *d*-orbital of platinum. There is evidence that properties of the *d*-orbitals of transition metals can be correlated with catalytic activity.[16] Furthermore, the coördination complexes of olefin–metal ion appear to involve the π-bond.[17] Thus in the platinum-catalyzed reduction of cholesterol the first step is the formation of the "platinomium" complex I (Fig. 2). This in turn is attacked by an activated deuterium atom or by a deuterium molecule coming from the van der Waals layer or from the solution. Because of steric factors at the ring juncture, the bond between the catalyst and substrate is displaced toward C-6 so that deuterium enters at C-5 to give the half-hydrogenated compound II. The half-hydrogenated II is further reduced to give cholestane-5,6-d_2-3β-ol (III) which is desorbed from the catalyst by the solvent. In the half-hydrogenated state II the catalyst at C-6 can readily dissociate the hydrogen on either C-5 or C-7 to form the "platinomium" complex I or IV. However, since the C-5 hydrogen is in a sterically unfavorable position, the half-hydrogenated compound II is more readily reduced to III or is in equilibrium with IV. The "platinomium" complex IV in turn is in equilibrium with deuterium and other species of half-hydrogenated states V and VII. Deuterium reduction of these complexes results in the formation of cholestanol (VIII) with deuterium atoms in positions 5, 6 and 7. The half-hydrogenated V can, in turn, equilibrate with the "platinomium" complex VI in which deuterium is both at C-5 and C-6. Further deuterium reduction of VI results in cholestanol IX with deuterium atoms at 5, 6α, 6β and 7. A combination of the 3 species of cholestanol III, VIII and IX can account for the isotope distribution found experimentally.

The exchange of hydrogen at C-6 during the course of platinum-catalyzed hydrogenation has been demonstrated by the converse of the reactions described above. When cholesterol-*d* with approximately 1 D at C-6[5] was reduced with platinum and hydrogen in non-isotopic acetic acid, the resultant cholestanol had lost 0.4 D *i.e.*, nearly half the deuterium on this carbon was capable of exchange with the medium in the presence of platinum and hydrogen. Since it has been demonstrated that no isotope enters C-4 during catalytic reduction, the result provides strong support for the existence of the "platinomium" complexes IV and VI. Inferentially, the equilibrium I ⇄ II must be displaced strongly in the direction of II.

The isotope distributed among C-5, C-6 and C-7 can be assigned to the individual carbon atoms if

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(15) G. H. Twigg, *Proc. Royal Soc. (London)*, **178A**, 106 (1941).

(16) F. C. Tompkins, *Ann. Reports*, **47**, 54 (1950); O. Beeck, *Disc. Faraday Soc.*, **8**, 118 (1950).

(17) J. Chatt, *J. Chem. Soc.*, 3340 (1949); J. Chatt and A. L. Duncanson, *ibid.*, 2939 (1953); A. D. Walsh, *ibid.*, 89 (1947).

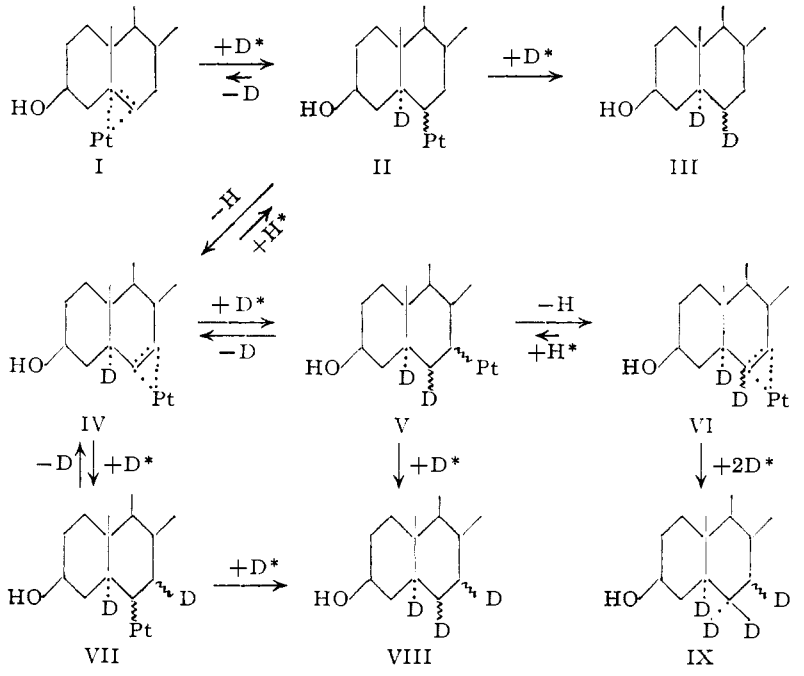
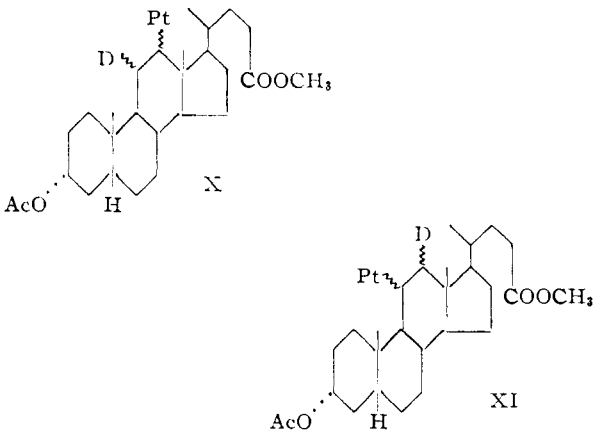


Fig. 2.

the reasonable assumption is made that the equilibrium V ⇄ VI is essentially that depicted in Fig. 2, or that the rate of V → VI is much faster than the hydrogenation steps V → VIII and VII → VIII. It was found experimentally that 0.4 D was located at C-7. It is therefore probable that an equal amount was at C-6 *in addition to the isotope at C-5 and C-6 resultant from the direct reduction of the half-hydrogenated state II.* Therefore it can be estimated that the cholestanol-$d_{2.55}$ obtained from the reduction of cholesterol contained 0.4 D at C-7, 1.27 D at C-6 (0.4 D + (1.75 D/2) and 0.87 D at C-5. It would be of interest to confirm this conclusion by reduction of analogously constituted steroids with the functional groups elsewhere in the molecule.

The fact that the double bond in methyl 3α-acetoxy-Δ[11]-cholenate was hydrogenated without exchange is readily explicable in terms of the foregoing mechanism. When the half-hydrogenated state X is formed, it cannot form a "platinomium" bond at C-13 since no protium is present at that position. The "platinomium" complex formation to C-9 from the half-hydrogenated state XI is hindered as attested by the very slow reduction of Δ[9(11)]-derivatives.[18] Consequently the further reduction of XI to the fully saturated methyl 3α-acetoxycholanate-11,12-d_2 is so much more rapid than "double bond migration" that no exchange or incorporation of deuterium at C-9 occurs.



The Role of the Solvent.—The solvent, CH_3COOD, plays an important part in the catalytic hydrogenation of the compounds studied. The reduction of cholesterol acetate in petroleum ether, octane, cyclohexane and ethyl acetate is extremely sluggish. Hershberg, *et al.*,[19] have shown that organic and mineral acids accelerate the catalytic reduction of cholesterol. It was found in the studies of isotope effect during catalytic hydrogenation of methyl 3α-acetoxy-Δ[11]-cholenate, that the reduction with D_2 in the presence of CH_3COOH proceeded with very little incorporation of isotope; conversely with H_2 in a medium of CH_3COOD, there was a large incorporation of isotope.[2] In the presence of active platinum there is a rapid exchange between the solvent and the dissolved gas and a slow equilibration between the hydrogen gas in the vapor phase. Therefore in solution, the isotope concentration in the solvent determines the incorporation of deuterium in a catalytic hydrogenation. However, it is unlikely that the proton (deuteron) in the acetic acid-*d* or from mineral acid is the active agent in the reduction. It is most likely that the proton (or acetic acid) acts as an effective desorbing agent for the reduced substance and thus accelerates the reaction as proposed by Farkas[20] for hydrogenation carried out in alcohol solution.

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