[CONTRIBUTION FROM THE DIVISIONS OF PHYSICS AND BIOPHYSICS AND OF STEROID BIOCHEMISTRY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Equilibrium between Protium, Deuterium and Tritium in the System: Hydrogen–Acetic Acid; Isotopic Fractionation Factors in a Catalytic Hydrogenation

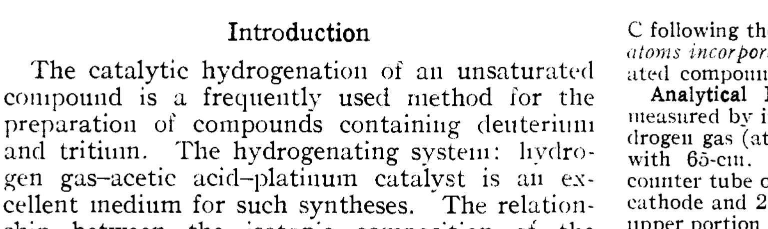
BY MAXWELL LEIGH EIDINOFF, JOSEPH E. KNOLL, DAVID K. FUKUSHIMA AND T. F. GALLAGHER[1]

RECEIVED MAY 12, 1952

Isotopic distribution coefficients in the equilibria at 25° of: H_2 + AcOH′ (l) → HH′ + AcOH (l) have been measured, where H′ refers to deuterium and tritium. Equilibrium constants were calculated for the corresponding gas reactions using these isotopic forms of acetic acid. This isotopically equilibrated hydrogenation system was used in the presence of platinum catalyst to saturate the double bond in methyl 3α-acetoxy-Δ^{11}-cholenate. The isotopic composition of the hydrogen atoms incorporated during the hydrogenation was measured relative to the isotopic composition of the carboxyl hydrogen of the acetic acid medium. These results indicate that the exchange between dissolved and carboxyl hydrogen at the catalyst surface is rapid relative to the hydrogenation steps. Relative to the hydrogen gas composition, the heavier isotopes are preferentially incorporated into the compound undergoing hydrogenation by a factor of 1.10 and 1.26 for deuterium and tritium, respectively. Relative to the carboxyl hydrogen composition, protium is preferentially incorporated by a factor of 3.31 and 5.42 with respect to deuterium and tritium, respectively.

Introduction

The catalytic hydrogenation of an unsaturated compound is a frequently used method for the preparation of compounds containing deuterium and tritium. The hydrogenating system: hydrogen gas–acetic acid–platinum catalyst is an excellent medium for such syntheses. The relationship between the isotopic composition of the hydrogenating medium and that of the hydrogen atoms incorporated into the compound that was reduced is of interest from the synthetic viewpoint as well as from the viewpoint of the isotopic fractionation factors involved. In order to provide information on these relationships, the hydrogen isotopic equilibrium has been studied at 25° in the system hydrogen–acetic acid using the isotopes of masses 1, 2 and 3 and the isotope distribution coefficients measured. The isotopic fractionation factors for these isotopes were also measured in the hydrogenation of a steroid, methyl 3α-acetoxy-Δ^{11}-cholenate (I), to form the corresponding cholanate (II).



I II

Experimental

Expression of Isotopic Ratios.—The isotopic concentrations of deuterium and tritium are expressed as atom ratios: (D/H) and (T/H). Thus, (D/H) refers to the ratio of the number of atoms of mass 2 to mass 1. The subscripts G and M following these atoms ratios refer to the gas phase over the solution and to the hydrogen atom in the *carboxyl group* of the acetic acid, respectively. Only this hydrogen atom is of interest in these studies because the methyl group hydrogen atoms do not exchange with the gas under the experimental conditions. The concentrations $(D/H)_G$ and $(T/H)_G$ were measured directly in the equilibrium studies while $(D/H)_M$ and $(T/H)_M$ were known. The subscript C following the isotopic atom fraction refers to the ***hydrogen atoms incorporated*** during the hydrogenation of the unsaturated compound (I).

Analytical Methods.—Relative tritium activities were measured by internal gas counting in which radioactive hydrogen gas (at approximately 5 cm. pressure) was admixed with 65-cm. methane gas. This gas mixture, inside a counter tube of about 18 mm. inside diameter with silvered cathode and 2 mil tungsten anode wire, was counted in the upper portion of the proportional region using a pulse amplifier and scaling circuit, the discriminator of which was set to accept all pulses greater than one millivolt.

Using this counting gas mixture, the characteristic curve had a plateau starting at 2900 volts, and extending for approximately 1000 volts with a slope of less than 0.3% per hundred volts. The counting rate is directly proportional to the partial pressure of the radioactive hydrogen gas and is independent of the methane gas pressure. At methane partial pressures of 20 cm., the plateau is shortened to 400 volts. The use of internal gas counting insures reproducible geometry. Resolving time corrections were made using the method described by Reid, Weil and Dunning.[2] A half-life of 12.1 years was used to correct all tritium activities for natural decay.[3] The measurement of relative tritium activities by this procedure is precise to within 1%. Water containing deuterium or tritium was first converted to hydrogen gas over zinc at 420° in a good vacuum. The activity of the hydrogen gas was then measured as described above. The isotopic analysis of the hydrogenated organic compound was made after complete combustion in a stream of dry oxygen with the resulting water collected in a trap cooled to −70°. The water was then converted to hydrogen over zinc. Memory effects were eliminated by discarding the product of the first combustion-conversion procedure and checking the results of a second and third run. The latter measurements were equal within 2%.

Deuterium measurements were made using a dual collector Nier type hydrogen mass spectrometer. Hydrogen gas for the latter was obtained from heavy water and the deuterium-containing compound in a manner similar to that stated above. The reliability of the deuterium measurements is 1–2% of the measured deuterium content. The tritium counting rates, corrected to a standard partial pressure inside the Geiger-Müller counter tube used in all the measurements are directly proportional to the (T/H) ratio in the hydrogen gas or organic compound. The mass spectrometer data yield (D/H) ratios directly since the (mass 3/mass 2) current ratio is compared with ratios for hydrogen samples made from heavy water samples of known deuterium content. Normal deuterium abundance was taken as 0.020%.

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[1] This investigation was jointly supported by the Office of Naval Research contract No. NC-ori-99, T.O. 1, the Atomic Energy Commission, and grants from the National Cancer Institute, United States Public Health Service.

(2) A. F. Reid, A. S. Weil and J. R. Dunning, *Anal. Chem.*, **19**, 824 (1947).

(3) A. Novick, *Phys. Rev.*, **72**, 972 (1947).

Reagents.—Methyl 3α-acetoxy-Δ^{11}-cholenate (I), was further purified by treatment with Raney nickel in ethyl acetate solution and filtration through Celite. The product melted 118–119° (reported[4] 116–117°), $[\alpha]^{25}\mathrm{D}$ $+53.5°$ (acetone) (reported[5] $+ 52.2°$). Platinum oxide catalyst (Baker and Co., Newark) was used without further purification. Heavy water (99.8%) and deuterium gas (99.6%) were obtained from the Stuart Oxygen Co. Oxygen was removed from tank hydrogen (Matheson Co.) by passage through a tube containing active platinum catalyst, trapping the water formed at Dry Ice temperature. Radioactive hydrogen gas was obtained from the Isotopes Division of the U. S. Atomic Energy Commission. Gas of lower activity was prepared by serial dilution of this material with oxygen-free tank hydrogen. The radioactive water used to prepare the acetic acid medium was prepared from hydrogen by reaction with copper oxide at 350°. The acetic anhydride (Eastman Kodak Co., 99–100%) was distilled in an all-glass 20-plate column packed with glass helices, and a middle fraction boiling at 140° at one atmosphere was used. Titration with sodium methylate and sodium hydroxide following the procedure of Smith and Bryant[6] showed that the acetic anhydride was free of acetic acid within 0.2%, the experimental error in this procedure.

The abbreviated formulas AcOD and AcOT refer to acetic acid containing deuterium and tritium in the hydrogen of the carboxyl group while the methyl hydrogens are of normal isotopic abundance. $(D/H)_M$ was approximately 0.03 and 1.0 for the equilibrium studies and hydrogenation experiments, respectively. $(T/H)_M$ was approximately 10^{-11} and 10^{-9}, respectively. The acetic acid medium was prepared by adding water to acetic anhydride in equimolar proportions. The isotopic composition of the carboxyl hydrogen of the acetic acid was thus equal to that of the water used in its preparation.

Measurement of Isotopic Equilibrium (Table I).—The glass-stoppered flask containing acetic acid, hydrogen gas and platinum catalyst (0.35, 8×10^{-3}, and 1.63×10^{-4} moles, respectively) was shaken vigorously in a thermostatic bath for approximately three hours. The hydrogen gas in isotopic equilibrium with the liquid was freed from acetic acid vapor by passage through a trap cooled in liquid nitrogen and the deuterium or tritium content measured as described above.

The isotopic composition of the carboxyl hydrogen of the acetic acid was practically constant throughout the shaking period, since the acid is present in large molar excess. Its composition at equilibrium was calculated from the known initial composition by correcting for the uptake of deuterium or tritium by the gas phase using the final isotopic composition of the gas and its volume at the measured temperature and pressure. This correction is of the order of 0.2%. An additional correction was made for the dilution of the isotopic content of the medium caused by the ordinary water, associated with the small quantity of platinum oxide catalyst used, the formula of which was considered to be $PtO_2 \cdot H_2O$. This correction is of the order of 0.2%.

Measurement of Isotopic Fractionation Factor (Table II).—Approximately 21, 0.5 and 0.04 g. of the acetic acid, steroid I and Adams platinum oxide catalyst, respectively, were shaken for 2.5 hours. This corresponds to molar proportions of acid, compound and catalyst equal to 300, 1 and 0.14, respectively. Approximately 0.006 mole of hydrogen gas were used in each experiment corresponding to an approximately 4.5-fold excess.

The hydrogenation reaction was completed in approximately 13 minutes, corresponding to the theoretical uptake of hydrogen gas within the experimental error of one per cent. The uptake was uniform for the first seven minutes, corresponding to 75% completion and was slower in the remaining six minutes.

At the end of the hydrogenation, the contents of the reaction flask were brought to Dry Ice temperature, the hydrogen gas was removed and the acetic acid containing deuterium or tritium was removed by distillation *in vacuo*. Residual quantities of the latter were removed by successive additions of ordinary acetic acid followed by vacuum distillation. The product II was freed of catalyst by filtration of the solution in acetone and purified by two fractional recrystallizations from petroleum ether. The melting point of the purified II was 134–135°. The tetranitromethane test for unsaturation gave uniformly negative results; $[\alpha]^{25}\mathrm{D}$ $+48.1°$ (acetone), (reported[5] m.p. 134°), $[\alpha]^{15}\mathrm{D}$ $+48.4°$. In preparations A, B, E and F of Table II, the reaction gas was first isotopically equilibrated with a large excess of acetic acid of isotopic composition equal to that used in the subsequent hydrogenation. The purpose of the prior equilibration was to minimize the effect caused by the changing isotopic composition of the gas phase during the hydrogenation. For preparations E, F and G, the $(D/H)_M$ was approximately 0.5 in order to furnish D/H ratios for the hydrogen from the combustion of II of approximately 0.01, a preferred concentration range for the mass spectrometer measurements. The acetic acid medium for preparations A to D contained approximately 10^{-9} atom fraction of tritium. In preparation C and G, the gas used had been equilibrated for a shorter time than would correspond to isotopic equilibrium. In preparation C, the gas used for the hydrogenation had attained slightly less than $^1/_4$ of the equilibrium T/H ratio. In preparation G, the gas composition was not measured. In preparation D, the hydrogen was of normal isotopic abundance at the start. The isotopic composition of the carboxyl hydrogen of the acetic acid medium was calculated as described above. The isotopic composition of the hydrogen atoms incorporated into II during the hydrogenation was calculated from the measured (D/H) and (T/H) composition of the hydrogen gas obtained from the combustion of II using its molecular formula and the assumption that two hydrogen atom positions were enriched in D or T while the other positions have the normal abundance of deuterium and no tritium.

In order to determine whether the methyl group of the acetic acid exchanged hydrogen during the reaction, silver acetate was prepared by treating a portion of the acetic acid medium after the hydrogenation. Tritium analyses of the hydrogen from the combustion of the silver acetate showed that such exchange did not take place to within the experimental uncertainty of 0.2%.

Results

A. Isotopic Equilibrium in the System H_2–Acetic Acid.—The approach to isotopic equilibrium in the reaction: $H_2 + AcOT(l) \rightleftarrows HT + AcOH(l)$ is shown in Fig. 1. The lower curve refers to an experiment in which tank hydrogen was shaken with AcOT containing a large molar excess of acetic acid relative to hydrogen gas. After two hours, the radioactivity of the hydrogen gas reached a constant value. The equilibrium position was approached in the opposite direction by starting

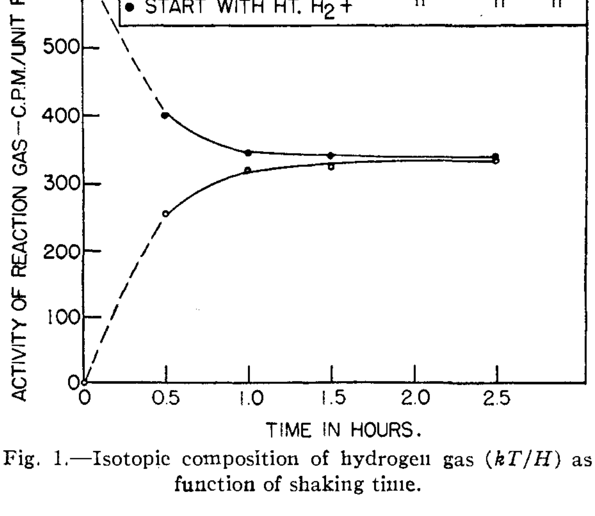

Fig. 1.—Isotopic composition of hydrogen gas (kT/H) as function of shaking time.

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[4] J. Press and T. Reichstein, *Helv. Chim. Acta*, **25**, 878 (1942).

[5] E. Seebeck and T. Reichstein, *ibid.*, **26**, 536 (1943).

[6] D. M. Smith and W. M. D. Bryant, This Journal, **58**, 2452 (1936).

with hydrogen gas containing more tritium than the equilibrium concentration (upper curve).

The approach to isotopic equilibrium in the reactions

$$H_2 + AcOD(l) \rightleftarrows HD + AcOH(l) \qquad (a)$$

$$H_2 + AcOH(l) \rightleftarrows HT + AcOH(l) \qquad (b)$$

was studied simultaneously by starting with a system of tank hydrogen and acetic acid containing atom fractions of deuterium and tritium equal to 0.03 and 10^{-11}, respectively. The gas isotopic composition relative to equilibrium is a measure of the approach to isotopic equilibrium and is plotted as ordinate in Fig. 2. The fractional approach to isotopic equilibrium as a function of time is not significantly different for the hydrogen–tritium and hydrogen–deuterium exchange.

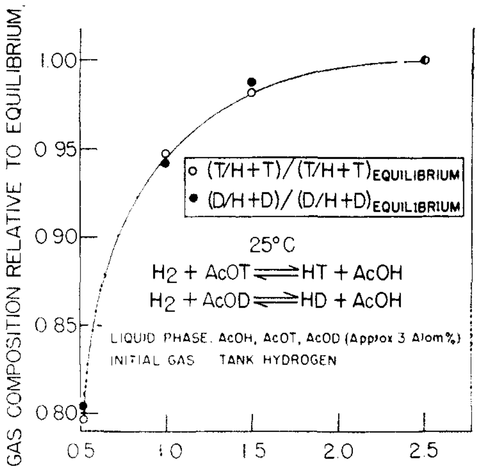

Fig. 2.—Approach to isotopic equilibrium.

The isotope exchange coefficient $\alpha_{H,D}$ for the reaction (a) is defined as: $\alpha_{H,D} = (D/H)_M/(D/H)_G$ where $(D/H)_M$ and $(D/H)_G$ are the atom ratios in the carboxyl hydrogen and hydrogen gas, respectively, at equilibrium. The average of experiments 2–4 in Table IA yields a value of 3.82_7 (a.d. ± 0.02_4) at 25.0°.

TABLE I

ISOTOPIC EXCHANGE COEFFICIENTS

$$(A)\ HD + AcOH\ (l) \rightleftarrows H_2 + AcOD\ (l)$$

$$(B)\ HT + AcOH\ (l) \rightleftarrows H_2 + AcOT\ (l)$$

Expt.	T, °C.	Shaking time (hours)	Isotopic exchange coefficient α	Remarks gas used
			A.	
1	25 ± 0.5	2.5	3.75 ± 0.07	Ordinary
2	25 ± .1	2	3.84 ± .07	hydrogen
3	25 ± .1	2	3.85 ± .07	used
4	25 ± .1	2	3.79 ± .07	Deuterium-rich gas
	Average of 2–4		3.82_7 (a.d., 0.024)	
			B.	
1	24.5 ± 0.5	2.5	6.88 (±0.1)	Ordinary hydrogen
2	24.5 ± .5	2.5	6.82 (± .1)	used
3	24.5 ± .5	2.5	6.86 (± .1)	Tritium-rich gas
	Average		6.85_3 (a.d., 0.023)	

The isotopic exchange coefficient ($\alpha_{H,T}$) for the reaction (b) is similarly defined as: $\alpha_{H,T} = (T/H)_M/(T/H)_G$ where $(T/H)_M$ and $(T/H)_G$ are the atom ratios in the carboxyl hydrogen and the hydrogen gas, respectively. The average of the three experiments in Table IB yields a value of 6.85_3 (a.d. $\pm 0.02_3$) at 24.5 ± 0.5°.

TABLE II

MEASUREMENT OF ISOTOPIC FRACTIONATION FACTOR DURING HYDROGENATION AT 25 ± 0.5°

Preparation	Isotopic composition of gas	Factor	
	$f_{H,D}$		
E	Equilibrated	3.35 ± 0.07	3.31_5 av.
F	Equilibrated	3.28 ± .07	
G	Partial equilibration	3.46 ± .07	
	$f_{H,T}$		
A	Equilibrated	5.41 ± 0.1	5.42 av.
B	Equilibrated	5.43 ± .1	
C	Partial equilibration	6.01 ± .1	
D	Tank gas	6.03 ± .1	

The isotopic exchange coefficient $\alpha_{H,T}$ (Table I) is constant over a wide range of tritium concentrations where the latter atom fraction is small relative to the concentration of light hydrogen. This is also the case during the hydrogenation experiments listed in Table II where the atom fraction of tritium is approximately 10^{-9}. This constancy of $\alpha_{H,T}$ may be shown by deriving a relationship between $\alpha_{H,T}$ and the equilibrium constant K_g(H,T) for the reaction

$$HT\ (g) + AcOH\ (g) \rightleftarrows H_2\ (g) + AcOT\ (g)$$

At moderate pressures

$$K_g\ (H,T) = \frac{p_{H_2}\ p_{AcOT}\ (g)}{p_{HT}\ p_{AcOH}\ (g)}$$

Assuming that the partial pressures of AcOH and AcOT follow ideal solution laws, it is calculated that

$$\alpha_{H,T} = 2K_g\ (H,T)\ \frac{p^\circ_{AcOH}}{p^\circ_{AcOT}} \text{ where} \qquad (1)$$

p°_{AcOH} and p°_{AcOT} refer to the vapor pressures of these components, respectively. The vapor pressure ratio AcOH/AcOT has not been measured.

The isotopic exchange coefficient $\alpha_{H,D}$ is a constant only at very low $(D/H)_M$ compositions, where it is related to the equilibrium constant K_g (H,D) for the reaction

$$HD(g) + AcOH(g) \rightleftarrows H_2(g) + AcOD(g)$$

by the equation

$$\alpha_{H,D} = 2K_g\ (H,D)\ \frac{p^\circ_{AcOH}}{p^\circ_{AcOD}}$$

At higher concentrations of deuterium, the $\alpha_{H,D}$ can be related to K_g (H,D) by using the equilibrium constant K' for the reaction

$$H_2 + D_2 \rightleftarrows 2HD$$

The general relationship is

$$\alpha_{H,D} = 2K_g\ (H,D)\ \frac{p^\circ_{AcOH}}{p^\circ_{AcOD}} \times \left[\frac{1 + (R(D/H)_M/2K_g(H,D))}{1 + (2R/K'K_g(H,D))(D/H)_M}\right] \qquad (2)$$

where $R = p^\circ_{\mathrm{AcOD}}/p^\circ_{\mathrm{AcOH}}$. K_g (H,D) may be calculated from equation (2) using the following data and literature values: $R = 1.012$,[7] $K' = 3.25$,[8] and $\alpha_{\mathrm{H,D}} = 3.83$ at $(\mathrm{D/H})_{\mathrm{M}} = 0.03485$. Using these values, K_g(H,D) at 25° = 1.94.

The vapor pressure of AcOT has not been measured. Since the vapor pressure ratio AcOT/AcOH probably does not differ from unity by more than three per cent., it may be assumed for the purpose of this calculation that the increase in vapor pressure of AcOT is double that of AcOD relative to AcOH. Using 1.024 for this ratio, K_g(H,T) may be calculated from equation (1) as 3.51 (±4%).

B. Fractionation Factors in Catalytic Hydrogenation.—The hydrogen gas–acetic acid–platinum catalyst system studied above was used for the hydrogenation of the double bond in methyl 3α-acetoxy-Δ^{11}-cholenate (compound I). It was observed that the isotopic composition of the medium $(\mathrm{T/H})_{\mathrm{M}}$ and $(\mathrm{D/H})_{\mathrm{M}}$ was of principal importance in determining the isotopic content of the hydrogenated compound II while the initial composition of the hydrogen gas, $(\mathrm{T/H})_{\mathrm{G}}$ and $(\mathrm{D/H})_{\mathrm{G}}$, was of minor importance. Consequently it was considered desirable to calculate a practical fractionation factor, f, where $f_{\mathrm{H,D}} = (\mathrm{D/H})_{\mathrm{M}}/(\mathrm{D/H})_{\mathrm{C}}$ and $f_{\mathrm{H,T}} = (\mathrm{T/H})_{\mathrm{M}}/(\mathrm{T/H})_{\mathrm{C}}$. Since the acetic acid was present in large molar excess, the values for $(\mathrm{D/H})_{\mathrm{M}}$ and $(\mathrm{T/H})_{\mathrm{M}}$ are essentially constant during the hydrogenation.

Several types of control experiments were run in order to check the absence of isotopic exchange during the hydrogenation which could invalidate the calculation of f. The methyl group of the AcOT did not incorporate tritium by isotopic exchange when treated with hydrogen gas and platinum catalyst under the hydrogenation conditions. Treatment of the saturated compound II with hydrogen gas and platinum catalyst in the AcOT medium showed that less than 1% of the tritium per atomic position was incorporated. Hydrogenation of I using deuterium gas (99.6%) and AcOD made from acetic anhydride and D_2O (99.8%) yielded in two such syntheses 2.02 ± 0.06 and 1.96 ± 0.06 gram atoms of deuterium per mole. From the average result 1.99 ± 0.06, it is concluded that, within experimental error two deuterium atoms are incorporated into II under the hydrogenation conditions in these experiments. Therefore, the calculations of the isotopic fractionation factors shown in Table II were made from the isotopic analyses of II on the basis that exactly two hydrogen atoms were incorporated during the hydrogenation.[9]

The fractionation factor results are shown in Table II. In the measurement of $f_{\mathrm{H,D}}$, the medium contained AcOD in which $(\mathrm{D/H})_{\mathrm{M}}$ was approximately unity. In the measurement of $f_{\mathrm{H,T}}(\mathrm{T/H})_{\mathrm{M}}$ was approximately 10^{-9}. Using the average of preparations E and F, it is seen that when hydrogen gas in isotopic equilibrium with the carboxyl hydrogen of the medium is used, light hydrogen is preferentially incorporated into I by a factor of 3.31 (±2%) relative to the isotopic composition of the carboxyl hydrogen. In preparation G, the hydrogen gas was richer in protium as a result of only partial equilibrium prior to the hydrogenation. The observed factor was shifted to 3.46 (±2%). Using the average of preparations A and B in the case of isotopically equilibrated gas, protium is preferentially incorporated into II by a factor of 5.42 (±2%) relative to the isotopic composition of the carboxyl hydrogen. In preparation C, the partially equilibrated gas was richer in light hydrogen than the equilibrium mixture. A factor of 6.01 ±0.1 was observed. In preparation D, tank hydrogen was used, resulting in the factor 6.03 ± 0.1.

The data for preparation F provide an additional check on the consistency of the fractionation factors $f_{\mathrm{H,D}}$ and $f_{\mathrm{H,T}}$ in Table II. The major purpose of this experiment was the direct determination of $f_{\mathrm{H,D}}$, and for this purpose the $(\mathrm{D/H})_{\mathrm{M}}$ was approximately one. The medium also contained an atom fraction of T approximately equal to 10^{-9}. The $(\mathrm{D/H})_{\mathrm{M}}$ was known; $(\mathrm{D/H})_{\mathrm{C}}$ was measured directly. $f_{\mathrm{H,D}}$ was 3.28. Tritium analyses of II permitted calculation of a quantity directly proportional to the atom fraction of tritium, $k\left[\frac{\mathrm{T}}{\mathrm{H+D}}\right]_{\mathrm{C}}$. The corresponding quantity, $k\left[\frac{\mathrm{T}}{\mathrm{H+D}}\right]_{\mathrm{M}}$, was known from the preparation of the acetic acid. The following equations were used to calculate $f_{\mathrm{H,T}}$ and $f_{\mathrm{D,T}}$

$$f_{\mathrm{H,T}} = (\mathrm{T/H})_{\mathrm{M}}/(\mathrm{T/H})_{\mathrm{C}} = \frac{\left(\frac{\mathrm{T}}{\mathrm{D+H}}\right)_{\mathrm{M}}\left(1+\frac{\mathrm{D}}{\mathrm{H}}\right)_{\mathrm{M}}}{\left(\frac{\mathrm{T}}{\mathrm{D+H}}\right)_{\mathrm{C}}\left(1+\frac{\mathrm{D}}{\mathrm{H}}\right)_{\mathrm{C}}}$$

and

$$f_{\mathrm{D,T}} = (\mathrm{T/D})_{\mathrm{M}}/(\mathrm{T/D})_{\mathrm{C}} = \frac{\left[\frac{\mathrm{T}}{\mathrm{D+H}}\right]_{\mathrm{M}}\left[1+\frac{\mathrm{H}}{\mathrm{D}}\right]_{\mathrm{M}}}{\left[\frac{\mathrm{T}}{\mathrm{D+H}}\right]_{\mathrm{C}}\left[1+\frac{\mathrm{H}}{\mathrm{D}}\right]_{\mathrm{C}}}$$

The experimental values for these quantities were $(\mathrm{D/H})_{\mathrm{M}} = 1.0355$, $(\mathrm{D/H})_{\mathrm{C}} = 0.316$; $\left(\frac{\mathrm{T}}{\mathrm{D+H}}\right)_{\mathrm{C}} = k'\ (7.04 \times 10^4)$; $\left(\frac{\mathrm{T}}{\mathrm{D+H}}\right)_{\mathrm{M}} = k'\ (2.45 \times 10^5)$. These values yield the following fractionation factors: $f_{\mathrm{H,D}} = 3.28$ (Table II); $f_{\mathrm{H,T}} = 5.38$; $f_{\mathrm{D,T}} = 1.64$. This value for $f_{\mathrm{H,T}}$ is within 1% of the average 5.42 in Table II. The isotopic composition of the carboxyl hydrogen in these two media was considerably different. In the former case, (H/D) was approximately one, while in the latter case, the deuterium was in normal abundance.

A value for $f_{\mathrm{D,T}}$ may be obtained from the results in Table II by dividing $f_{\mathrm{H,T}}$ by $f_{\mathrm{H,D}}$

$$f_{\mathrm{D,T}} = \frac{(\mathrm{T/H})_{\mathrm{M}}(\mathrm{T/H})_{\mathrm{C}}}{(\mathrm{D/H})_{\mathrm{M}}(\mathrm{D/H})_{\mathrm{C}}} = 5.42/3.31_5 = 1.63_5$$

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[7] J. O. Halford and L. C. Anderson, THIS JOURNAL, **58**, 736 (1936); Lange's Handbook, 5th edition.

[8] H. C. Urey, J. Chem. Soc. 562 (1947).

[9] Our analytical data do not preclude the possibility that a small amount of isotopic exchange takes place in the immediate vicinity of the 11,12-positions during the hydrogenation. For this reason, reference has been made above to the isotopic composition of the hydrogen atoms incorporated in II during the hydrogenation in preference to the use of the phrase hydrogen atoms entering the double bond during the reduction.

The isotopic fractionation factors listed in Table II have been defined relative to $(D/H)_M$ and $(T/H)_M$. This definition was used since, as has been shown, the isotopic composition of the medium dominates the $(D/H)_C$ and $(T/H)_C$ in the hydrogenated compound II. The usefulness of this definition has been shown by noting the consistency of the $f_{D,T}$ measured in one experiment with the $f_{H,T}$ and $f_{H,D}$. On this basis, it is seen that relative to the medium, H is preferentially incorporated with respect to T by the factor 5.42 and with respect to D by the factor 3.31_5 at 25°.

It has been shown from the results of experiment D in Table II that the isotopic composition of the medium determines $(T/H)_C$ and $(D/H)_C$. It may therefore be assumed that the hydrogen gas, in isotopic equilibrium with the medium at the start, remained in isotopic equilibrium throughout the hydrogenation. Under these conditions, the gas compositions $(T/H)_G$ and $(D/H)_G$ would be essentially constant, since $(T/H)_M$ and $(D/H)_M$ remain unchanged. It was therefore possible to express the isotopic fractionation factors with respect to the gas–compound II system as

$$f'_{H,T} = (T/H)_C/(T/H)_G = \alpha_{H,T}/f_{H,T} \qquad (3)$$

At 25°, $f'_{H,T} = 1.26$ when the previously determined values 6.85_3 and 5.42 for $\alpha_{H,T}$ and $f_{H,T}$ are substituted in equation 3. This calculation indicates a preferential incorporation of the heavier isotope relative to the equilibrium gas by the factor 1.26. The corresponding factor

$$f'_{H,D} = (D/H)_C/(D/H)_G = \alpha_{H,D}/f_{H,D} \qquad (4)$$

can be calculated in a similar manner. When (D/H) is equal to one, $\alpha_{H,D}$ is 3.66 (eq. 2). Using $f_{H,D}$ equal to 3.31_5, the calculated value for $f'_{H,D}$ is 1.10. This result again shows a slight preferential incorporation into (II) of the heavier isotope relative to the gas composition $(D/H)_G$.

Discussion

Our experiments have shown that the isotopic composition of the carboxyl hydrogen is of principal importance in determining the isotope ratios for the hydrogen atoms incorporated during the reduction. This was observed in the preparation D (Table II) where the $f_{H,T}$ increased slightly to 6.03 when tank hydrogen containing no tritium was used in the reduction. This dominant influence of the medium was confirmed by reducing I using ordinary acetic acid and 99.6% deuterium gas. Despite this high concentration of deuterium in the gas at the start, the ratio of deuterium to light hydrogen in the hydrogen atoms incorporated into II during the hydrogenation was found to be less than 0.01. These results support the hypothesis that isotopic exchange between dissolved hydrogen and carboxyl hydrogen or hydrogen ion is rapid relative to the reduction steps. As a result of this isotopic exchange, the isotopic composition of the dissolved gas rapidly attains a value related to that of the medium by the exchange coefficients, α, (Table I). The use of a different medium can yield different relative rates of exchange and reduction. For example, Rittenberg, *et al.*,[10] found that the catalytic reduction is faster than the isotopic exchange between the hydrogen and water in the catalytic hydrogenation of maleic acid in aqueous solution using active platinum or palladium.

Our experiments have shown also that the isotopic composition of the hydrogen atoms incorporated into II during the reduction is not equal to that for the carboxyl hydrogen. Taking the latter isotopic composition as a basis, it was found that light hydrogen was preferentially incorporated by a factor of 3.31 and 5.42 relative to deuterium and tritium, respectively. These practical fractionation factors $f_{H,T}$ and $f_{H,D}$ are of interest in connection with the synthesis of tracer compounds containing T and D for which the conditions and mechanisms are similar to those for the reaction outlined above. In tracer metabolism studies, it is sometimes desirable to obtain the labeled compound with as high a specific activity as possible. In the experiments described above, the reduced compound II is diluted with respect to the heavier isotopes in accordance with the fractionation factors $f_{H,D}$ and $f_{H,T}$.

That the heavier isotopes are preferentially incorporated into I by a factor of 1.10 and 1.26 for deuterium and tritium, respectively, *relative to the hydrogen gas composition* is of interest, since the lighter isotopes are known to react more rapidly in many reactions that involve the rupture of a relatively strong valence bond. This was confirmed recently in the photochlorination of $CHCl_3$[11] and the oxidation of succinic acid in the presence of the succinic acid oxidase enzyme system.[12] Bigeleisen,[13] following a general treatment of the influence of the partition functions of the reacting molecules and the activated complex on relative isotopic rates showed that the order of rate constants for reactions involving free hydrogen atoms as reactants will be $k_T > k_D > k_H$. Polanyi[14] reached a similar conclusion after consideration of zero point energies for initial and activated state. These considerations will be applicable to the results of these experiments if it is assumed that the reacting hydrogen atoms are relatively loosely bound at the catalyst surface,[15] so that isotopic zero point energy differences here are small. The isotopic fractionation factor would also be affected by the isotopic exchange coefficient between hydrogen in the gas phase and at the catalyst surface.

Acknowledgments.—We wish to express our appreciation to Robert W. Jailer for carrying out the deuterium analyses and to Dr. Harold Beyer and Mones Berman who furnished valuable assistance in connection with the deuterium mass spectrometer.

NEW YORK, N. Y.

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