methane. Concentration to dryness and crystallization from ether furnished 75 mg. of Ia-4-C^{14}. The remaining acidic residues were esterified similarly and added to a pool consisting of all residual fractions remaining after isolation of Ia-4-C^{14}. Treatment with an equal weight of sodium hydroxide (aqueous 6 *N*) in 50 parts of methanol for 1.5 hours at room temperature gave a neutral fraction (1.16 g.) from which there separated an additional 0.323 g. of labeled ester Ia, m.p. 126–132°.

The total yield of radioactive Ia based on 148 mc. of methyl iodide-C^{11} was 9.831 g., 29.75 mmoles, 59.8%.

Progesterone-4-C^{14} (Ic-4-C^{14}).—A solution of 5.4026 g. of Ia-4-C^{14} in 90 ml. of methanol and 13.5 ml. of aqueous 6 *N* potassium hydroxide was refluxed for 6 hours.[17] After the usual work-up, there resulted 4.127 g. of unpurified Ib-4-C^{14}, m.p. 225–230°, 95.7%. This material was diluted with inactive acid Ib[18] to a total weight of 8.422 g. (calculated activity, 1.47 mc. per mmole).

The sodium salt of Ib-4-C^{14} was prepared by a modification of the method of Wilds and Shunk[10] employing 0.95 equivalent of methanolic sodium hydroxide. The acid chloride, prepared in ether solution[19] by means of oxalyl chloride, was treated with excess diazomethane[13] to afford 21-diazoprogesterone-4-C^{14}, 4.39 g., m.p. 178–180° dec., and 3.76 g., m.p. 174–176° dec.[10] The yield of 21-diazoprogesterone-4-C^{14} was 90.1% based on Ib-4-C^{14}. A second Arndt–Eistert reaction using 9.711 g. of Ib-4-C^{14} (calculated activity, 1.47 mc. per mmole.), gave an 89.1% conversion to 21-diazoprogesterone-4-C^{14}.

Treatment[6] of the above diazoketone with 47% hydriodic acid gave an 87% yield of crude progesterone-4-C^{14} (Ic-4-C^{14}), m.p. 120–128°. Recrystallization from ether (activated charcoal) afforded 11.814 g., m.p. 127–131°, $[\alpha]^{21}D$ 203 ± 3° ($CHCl_3$), 37.2% based on methyl iodide-C^{14}. The specific activity per mmole. was found[20] to be 1.52 mc.; calculated activity 1.47 mc. A portion (5.459 g.) of this material was freed from a final trace of color by chromatography over untreated alumina (Alcoa F-20) to give an 81% recovery of pure progesterone-4-C^{14} (Ic-4-C^{14}), m.p. 132–133.5°, $[\alpha]^{21}D$ 202 ± 2° ($CHCl_3$), $\lambda_{max}^{ethanol}$ 240 mμ (ε 16680).

(18) The variability of the reported constants for this substance has prompted us to record those of a purified sample. Following several crystallizations from chloroform and then ethyl acetate, the data were: m.p. 253–257°, $[\alpha]^{25}D$ 165° ($CHCl_3$) and $\lambda_{max}^{ethanol}$ 238.5–240 mμ (ε 17750).

(19) C. H. Gleason, unpublished data from these laboratories.

(20) R. D. H. Heard, L. Thompson and C. Yates, *Proc. Can. Physiol. Soc.*, 23 (1951).

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Platinum-catalyzed Exchange of Hydrogen Isotopes with Bile Acids

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In the application of stable hydrogen isotope tracer techniques to bile acids, it is necessary to introduce deuterium into positions where it will remain stably bound under the conditions employed in the tracer experiments. We have found a method for obtaining as many as eight stably bound atoms of deuterium per molecule of either desoxycholic acid or cholic acid. This constitutes a considerable improvement over the results reported thus far with platinum-catalyzed hydrogen isotope exchange on steroids in deuterioacetic acid.[1–5] Our departure from the procedure of previous workers is in the use of deuterium oxide and alkali as the reaction medium for the platinum-catalyzed exchange.

(1) K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943).

(2) H. S. Anker and K. Bloch, THIS JOURNAL, **66**, 1752 (1944).

(3) D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 861 (1952).

(4) D. K. Fukushima and T. F. Gallagher, *ibid.*, **198**, 871 (1952).

(5) J. Bell and S. J. Thomson, *J. Chem. Soc.*, 572 (1952).

Experimental

Desoxycholic acid was purified by refluxing with carbon tetrachloride (1 liter per 20 g. of acid), filtering while hot, drying *in vacuo* at 25°, recrystallizing from acetone and drying *in vacuo* at 155°.

The reaction vessel used in the deuterization consisted of a narrow-necked 100-ml. Pyrex cylinder which fitted by means of a ground glass joint into a vapor jacket equipped with side arms for boiler and condenser. Both were flat-bottomed to permit magnetic stirring of the reaction mixture in the sealed cylinder. Methyl cellosolve was used to maintain the temperature at 124°.

Platinum catalyst was prepared by reduction of platinum dioxide with hydrogen in water suspension, followed by several washes with deuterium oxide to remove the water and to replace the hydrogen sorbed on the catalyst surface with deuterium.

A typical reaction mixture consisted of 10 g. of pure desoxycholic acid (m.p. 174–175°), 50 ml. of deuterium oxide (99.8%) containing 2 g. (2.05 equiv.) of sodium hydroxide and the platinum obtained by reducing 1.5 g. of platinum dioxide as described above. After the introduction of a glass-covered stirring bar, the reaction cylinder was flushed with nitrogen and sealed.

The reaction was allowed to proceed at 124° with stirring for seven days. After removal of the catalyst by centrifugation, the solution was diluted with 125 ml. of distilled water and acidified with hydrochloric acid to precipitate the deuterized desoxycholic acid. The product was filtered off, washed with water until chloride-free, dried *in vacuo* at 25° and crystallized twice from acetone, using 4 g. of Darco G60 the first time. The crystals were dried *in vacuo* at 155°; yield 3.3 g. of deuteriodesoxycholic acid, m.p. 173–174°, atom per cent. deuterium 18.4. The deuterium content was *unchanged* by refluxing one-half hour in methanol containing 5% potassium hydroxide. Repeat experiments gave materials containing 17.8, 13.7 and 20.0 atom per cent. deuterium, respectively. Further experiments with different amounts of catalyst and for extended periods of time have provided no unequivocal evidence as to the causes for this variation in extent of deuterium exchange. Fluctuations in temperature, poisoning of the catalyst by degradation products or by extraneous impurities may all be factors.

Cholic acid was submitted to deuterium exchange in a similar manner. However, cholic acid was found to differ markedly from desoxycholic acid in its susceptibility to degradation. Whereas only small amounts of by-products were formed in the case of desoxycholic acid, the side-reactions in the case of cholic acid consumed about half the starting material. This is what might be expected in view of the well-known lability at the 7-hydroxyl position in this substance. A more elaborate purification process was, therefore, required. Based upon preliminary experiments which included a chromatographic study of the crude reaction product, an isolation procedure was devised which is illustrated by the following example. Thirty grams of cholic acid was treated in a stainless steel autoclave with 180 ml. of deuterium oxide containing 6.5 g. (2.3 equiv.) of sodium hydroxide. Seven grams of a 10–13% platinum-on-charcoal catalyst was added. The reaction was carried out under nitrogen for 2 days at 115° with stirring. After cooling, the catalyst was removed by filtration. The filtrate was acidified with 75 ml. of 2.5 *N* hydrochloric acid and 10 g. of sodium sulfate was added. This solution was extracted three times with an equal volume of a mixture containing 1 part isopropyl alcohol to 2 parts ethyl ether. The combined non-aqueous phase was washed and dried over anhydrous sodium sulfate, then evaporated to dryness, with occasional small additions of acetone to facilitate removal of the isopropyl alcohol. The residue was a brown gummy mass containing 20.0 atom per cent. deuterium. This residue was taken up in acetone and treated with an excess (about 7 g.) of fresh diazomethane in ether. After the esterification was complete, excess ether was removed and enough acetone was added to make the total volume equal one liter. This acetone solution was charged on to a chromatographic column containing 400 g. of dry alumina, acid-washed to neutrality. The charged column was then washed

with about 3.5 liters of acetone to remove impurities. Successful removal of impurities was indicated by the fact that crystalline cholic acid could be obtained easily from the last 50 ml. of acetone wash by evaporation to dryness and scratching. (The rate of both charging and washing the column was maintained at not greater than 3 ml. per min.) The methyl cholate was then eluted with about 2.5 liters of absolute methanol. This eluate was evaporated to 100 ml., seeded with pure methyl cholate and crystallized at 5° for 24 hours. The filtered and dried crystals (4.5 g.) were saponified by dissolving in 100 ml. of 0.8 *M* aqueous sodium hydroxide solution and heating for one hour on the steam-bath. This solution was acidified with 50 ml. of 2.5 *N* hydrochloric acid and, after addition of 5 g. of sodium sulfate, was extracted with an equal volume of a mixture containing one part isopropyl alcohol to three parts ethyl ether. The non-aqueous phase was treated as the previous similar solution of crude material, *i.e.*, washed, dried over anhydrous sodium sulfate and evaporated to dryness. Final crystallization of the residue from acetone yielded 3.6 g. of deuterocholic acid containing 20.0 atom per cent. deuterium, m.p. 199–204°. On the basis of frontal analysis, we believe the purity of this material to be above 97%; we attribute the wide melting point range to polymorphism.

Analysis for deuterium content was carried out by combustion of the steroid in a stream of dried oxygen and measurement of the infrared absorption[6,7] of the condensed water of combustion at 3.198 μ, diluting if necessary with ordinary water to bring the deuterium oxide concentration into measurable range. Infrared measurements were made with a model 12C Perkin–Elmer infrared spectrophotometer.

Our interest in these exchange reactions has arisen from the need for stably labelled materials in quantitative analysis by the isotope dilution method.[8,9]

In the light of the findings of van Heyningen, Rittenberg and Schoenheimer[10] concerning the hydrogen–deuterium exchange in the fatty acids under the influence of alkali and platinum catalyst, it seems reasonable to assume that a large proportion of the hydrogen atoms exchanged with the bile acid takes place along the fatty acid side chain. There is evidence, however, which we have obtained since the subject matter of this paper was completed which indicates that some of the observed exchange takes place on the steroid polycyclic nucleus probably at positions alpha to the oxygen-bonded carbon atoms (exclusive of the spontaneously labile hydrogens of the hydroxyl groups). In any case whatever their positions all the deuterium atoms introduced are relatively stable ones since they have been demonstrated not to be labile in the absence of the platinum catalyst.

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(6) V. Thornton and F. E. Condon, *Anal. Chem.*, **22**, 690 (1950).

(7) N. R. Trenner and R. W. Walker, *The Perkin–Elmer Instrument News*, **4**, 1, (1952).

(8) N. R. Trenner, R. W. Walker, B. Arison and R. P. Buhs, *Anal. Chem.*, **21**, 285 (1949).

(9) N. R. Trenner, R. W. Walker, B. Arison and C. Trumbauer, *ibid.*, **23**, 487 (1951).

(10) W. E. van Heyningen, D. Rittenberg and Rudolf Schoenheimer, *J. Biol. Chem.*, **125**, 495 (1938).

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The Conversion of 17α,21-Dihydroxypregnane-3,20-dione to 3α,17α,21-Trihydroxypregnan-20-one *in vitro*[1,2]

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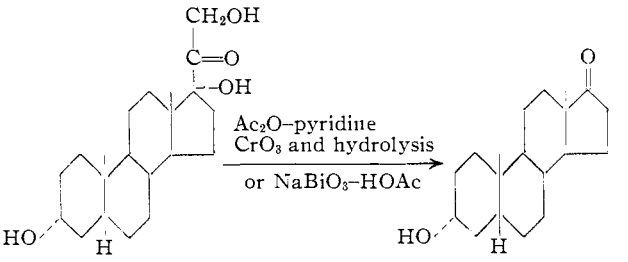
A possible intermediate in the metabolism of Reichstein's substance S, 17α,21-dihydroxypregnane-3,20-dione (dihydro S), has been administered to a female rheumatoid arthritic patient, and 3α-hydroxyetiocholan-17-one, 3α,17α,20α-pregnanetriol and 3α,17α,21-trihydroxypregnan-20-one (tetrahydro S) have been isolated from the urine.[3] The diverse reactions indicated by the *in vivo* study have been investigated in more detail using various *in vitro* systems. This report is concerned specifically with the conversion of dihydro S to the tetrahydro derivative, 3α,17α,21-trihydroxypregnan-20-one, by incubation with a rat liver homogenate.

The tetrahydro S obtained from the urine following the administration of dihydro S *in vivo* was identical to the product of the incubation with rat liver homogenate. The product of the incubation was detected, using the paper chromatographic technique,[4] with the triphenyltetrazolium chloride reagent. The compound migrated 0.12–0.17 cm./hr. in the toluene–propylene glycol system and the dihydro S starting material migrated 1.2–1.5 cm./hr. The structure of the tetrahydro S was established by the data given in Fig. 1. Chromic acid



3α,17α,21 - Trihydroxypregnan - 3α - Hydroxyetiocholan - 17 - 20-one: free, m.p. 214–216°; $[\alpha]^{27}D$ +60° (ethanol); one: diacetate, m.p. 201–206°, $[\alpha]^{27}D$ +77° (ethanol)

	alc. M_D	ΔM_D
3α,5β-THS diacetate	+338°	
3α,5β-THS free	+212°	+126°
3β,5β-THS diacetate	+228°	
3β,5β-THS free	+190°	+38°

Fig. 1.

oxidation of the tetrahydro S diacetate followed by hydrolysis, and oxidation of the tetrahydro S with $NaBiO_3$, both yielded 3α-hydroxyetiocholan-17-one. A comparison of the molecular rotational differences (ΔM_D) of the free 3α,5β-tetrahydro S and its diacetate with that of the free 3β,5β-tetrahydro S and its diacetate, showed a large positive increase for the 3α- as compared to the 3β-derivative.[5] The reduction of the 3-ketone to the 3α-hydroxy group with rat liver homogenate proceeded to the extent of 60–70%. In contrast, only minute amounts of tetrahydro S were isolated from the urine in the *in vivo* study. Dihydro S was converted to the 3α,5β-tetrahydro S by incubation with rabbit liver homogenate and rabbit kidney homogenate, the latter conversion proceeding to a lesser extent. The reduction to the 3α,5β-tetrahydro S with rat liver slices also proceeded to the extent of 60%. In this case, also, a small amount of the 3β,5β-tetrahydro S (0.5%) was isolated.

The 3α-hydrogenase of rat liver has been charac-

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(2) Presented at the 19th International Physiological Congress, Montreal, Canada, September 1–4, 1953.

(3) F. Ungar, J. W. Davis, H. Rosenkrantz and R. I. Dorfman, *J. Biol. Chem.*, in press for March 1954.

(4) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

(5) D. H. R. Barton and W. Klyne, *Chem. & Ind.*, 755 (1948).