The Preparation of [16-^{3}H]Progesterone

By W. H. PEARLMAN
Guy's Hospital Medical School, London, S.E. 1

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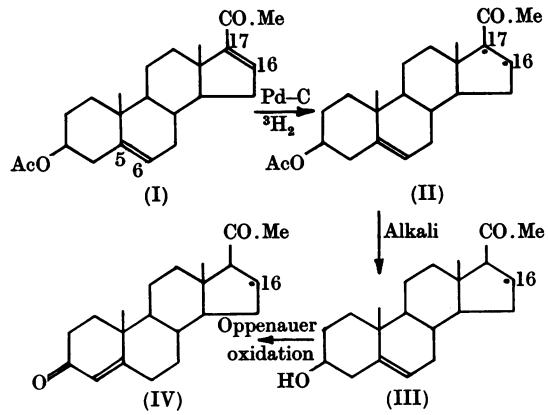
Radioactive progesterone of very high specific activity was required for certain *in vitro* experiments and at appropriate dilutions for *in vivo* studies on the metabolism of the hormone. It seemed likely that such a product might be prepared with comparative ease and economy by utilizing tritium as the isotopic labelling agent. Catalytic hydrogenation of an unsaturated steroid is a simple procedure and a large amount of hydrogen isotope can be introduced by this means. The double bond must be appropriately located in the molecule if the isotope is to be stably bound. Inasmuch as progesterone had been prepared from 3β-hydroxypregna-5:16-dien-20-one [commonly referred to as 16-dehydropregnenolone, unacetylated (I)] by differential catalytic hydrogenation, followed by Oppenauer oxidation (Marker *et al.* 1947), this approach to labelling progesterone with tritium appeared highly feasible, although only half of the tritium which might be initially introduced into ring *D* could be expected from theoretical considerations to be stably bound. The results obtained with the acetate of 16-dehydropregnenolone (I), which is commercially available and inexpensive, are described below.

EXPERIMENTAL

General. Melting points were determined on a Kofler-block type of apparatus and are corrected. *cyclo*Hexane, toluene and ether were stored over Na wire and redistilled over CaH_2 just before use. Light petroleum (AnalaR, b.p. below 40°) was treated with conc. H_2SO_4 in a separating funnel, carefully washed with water, dried over CaH_2 and distilled. The Al_2O_3 was Spence 'Type H' (Peter Spence, Widnes); it was treated with acetic acid, exhaustively washed with water and dried at 150° overnight.

Tritiation apparatus and procedure. The design of the apparatus was based on the one described by Berstein, Bennett & Fields (1952). The air in the system was displaced with N_2; tritium diluted with the theoretically required volume of H_2 was then introduced. The N_2 and H_2 were dried by passage over $Mg(ClO_4)_2$. A 10% Pd–charcoal catalyst was prepared by a hydrogenation procedure, as described by Fieser (1941); a magnetic stirrer afforded vigorous agitation of the reaction mixture which occupied about three-quarters of the reaction vessel. The progress of the reaction, which was carried out at atmospheric pressure and at room temperature, was followed with the aid of a mercury manometer, which had also served as a Toepler pump for the introduction of tritium into the system. When the reaction was over, the system was flushed with N_2; the entire process was carried out in a well-ventilated fume cupboard.

Determination of tritium. A weighed amount of steroid (containing about 0·03 μc of tritium) was dissolved in toluene (1·5 ml.) containing 0·5% (w/v) of the phosphor, *p*-terphenyl. The solution was transferred to an optical cup for measurement in a liquid scintillation counter (Avivi, Simpson, Tait & Whitehead, 1954) which had a detection efficiency for tritium of about 7%. A solution of a steroid of known specific activity was employed on each occasion as a reference standard; sufficient counts were recorded to ensure a standard deviation of $\pm 3\%$.



The sign • indicates localization of the isotope; a small amount of isotope may also be located at C-15, as discussed in the text.

Preparation of tritium-labelled steroids

[16:17-$^3H_2$]-3β-*Hydroxypregn-5-en-20-one acetate* (*II*). 3β-Hydroxypregna-5:16-dien-20-one acetate (I) (503 mg., 1·41 m-moles), m.p. 173–174°, was freed from any ethanol of crystallization before use by dissolving in *cyclo*hexane, evaporating to dryness *in vacuo*, and further drying in a vacuum desiccator over $CaCl_2$. The 5:16-diene (I) was redissolved in *cyclo*hexane (80 ml.), 10% Pd–charcoal catalyst (500 mg.) was added and the mixture stirred in a tritium–H_2 atmosphere (nominally 100 mc of tritium; H_2 was in slight excess of the theoretical requirement); the gas uptake ceased after 2 hr. (1·63 m-moles, corrected for the uptake by the catalyst; theory, 1·41 m-moles). The catalyst was removed by filtration and washed with ethanol. The combined filtrates were brought to dryness, and the residue, after three crystallizations from ethanol, gave a product (238 mg.), m.p. 150–151°; $[\alpha]_D^{22} + 13{\cdot}4 \pm 0{\cdot}8°$ in $CHCl_3$

(*c*, 5·01); specific activity 57·5 μc/mg. or 20·6 mc/m-mole. A second crop (106 mg.) of the same product was obtained on reworking the mother liquors. In all, the yield was 88%, representing a 20% radiochemical yield.

[16-^{3}H]-3β-*Hydroxypregn-5-en-20-one* (*III*). The [16:17-$^3H_2$]acetate (II) (226 mg.) was dissolved in a methanol solution (15 ml.) containing 5% (w/v) KOH and 20% (v/v) water. After refluxing for 3 hr., the solution was diluted with water and extracted with ether. The ether extract was washed with water and brought to dryness. The residue was crystallized twice from ethanol to yield a product (105 mg., 53%), m.p. 181–183°; specific activity 26·9 μc/mg. or 8·48 mc/m-mole. The percentage of tritium retained in the steroid molecule was 41.

[16-^{3}H]*Progesterone* (*IV*). [16-^{3}H]Pregnenolone (III) (104 mg.) was dissolved in dry toluene (50 ml.) and freshly distilled *cyclo*hexanone (7·5 ml.) was added. About 10 ml. of liquid was distilled to dry the system and then aluminium *iso*propoxide (300 mg.) was added. The reaction mixture was refluxed for 1 hr. and then steam-distilled, about 250 ml. of distillate being collected. The oily product was acidified with dil. HCl and extracted with ether. The ether extract was washed with 5% (w/v) Na_2CO_3 and finally with water. It gave on evaporation a partly crystalline product (119 mg.) which was purified by chromatography on Al_2O_3 (5 g.) with light petroleum–ether (3:1, v/v) as eluent. The crystalline product (94 mg.) was recrystallized from ether–light petroleum to afford prisms (57 mg., 55%), m.p. 128–129°; $[\alpha]_D^{23}$ +184±6° (*c*, 0·5); light-absorption max., 2400 Å (ε 16 650); specific activity 28·8 μc/mg. or 9·06 mc/m-mole.

The series of reactions were repeated but starting with 168 mg. (0·47 m-mole) of acetate (I) and 2·0 c (nominal) of tritium; [16-^{3}H]progesterone was obtained with specific activity 1·60 mc/mg. or 503 mc/m-mole (expected 1·73 mc/mg. on the basis of the results obtained in the previous run).

DISCUSSION

About one-fifth of the available tritium was incorporated into the steroid molecule in the initial step (catalytic reduction of I). To account for this discrepancy, the following factors, among others, might be mentioned: (1) catalytic exchange of hydrogen atoms between the gas phase and the solvent; (2) the relative roles of the gas phase and the solvent as donors of hydrogen atoms to the unsaturated steroid substrate; (3) the difference in the rates of reaction of hydrogen ($^1H_2$) and tritium which are ascribed to the difference in the effective mass of the isotope and hence of its 'zero point' energy; this may lead in certain instances to isotope fractionation (discussed by Kamen, 1951). The first factor may be the most important, since Farkas & Farkas (1939) had demonstrated a catalytic exchange of hydrogen atoms between molecular deuterium and the non-polar solvent, *cyclo*hexane. However, this exchange is very slow by comparison with that which obtains with hydroxylic solvents. The extent to which tritium may have entered into the *cyclo*hexane molecule was not determined in the present instance; the experimental conditions were not strictly comparable with those described by Farkas & Farkas (1939), who employed a platinized platinum foil and a hydrogen isotope of smaller mass. Incidentally, it should be noted that the acetate (I) was employed rather than the free hydroxyl compound, because catalytic exchange with this group would be expected to occur readily; also, that the 5:6 double bond resists reduction even in the presence of excess of hydrogen with palladium charcoal as catalyst.

Catalytic reduction of a double bond with hydrogen isotopes does not necessarily result in the localization of the isotope on the two carbon atoms. For example, Fukushima & Gallagher (1955) found that on catalytic reduction with deuterium of the 5:6 double bond of cholesterol acetate, about one-sixth of the total amount of deuterium introduced into the steroid molecule was located at C-7 and the remaining five-sixths equally distributed between C-5 and C-6. To account for this distribution of isotope, a mechanism was proposed involving the formation of a complex between the catalyst and the steroid molecule at C-5 and C-6 and its subsequent displacement toward the adjacent carbon, C-7. In view of these observations, it is conceivable that catalytic reduction of the 16:17 double bond of (I) may have led to the introduction of a small amount of tritium at C-15 with the major amount distributed between C-17 and C-16. Tritium at C-17 was not expected to be stably bound because C-20 ketones are known to undergo enolization, probably with the formation of a 17:20 double bond. Thus, Butenandt & Fleischer (1937) observed that 3β-hydroxypregn-5-en-20-one can be isomerized in part at the asymmetric centre (C-17) adjacent to the carbonyl group at C-20 by the action of 5% methanolic potassium hydroxide; the reactions are reversible and equilibrium is reached at a ratio of 70 parts of the normal ketone (C 17-β) to 30 parts of the 17-*iso*ketone (C-17α). In the present work, prolonged refluxing of the [16:17-^{3}H]acetate (II) with alkali resulted in a loss of 59% of the isotope content of the molecule.

It may be possible to prepare [16-^{3}H]progesterone of specific activity even higher than that described by utilizing 97% tritium gas and a few milligrams of the starting steroid (I); a micro-tritiation apparatus on a 1 ml. scale has been described by Glascock (1954).

Other methods for labelling progesterone with hydrogen isotope have been described. Koechlin, Kritchevsky & Gallagher (1950) prepared [11:12-$^2H_2$]-progesterone from methyl [11:12-$^2H_2$]lithocholate; the latter was obtained on reduction of methyl 3-acetoxy-11:12-cholenate in $CH_3 \cdot CO_2{}^2H$ with deuterium gas in the presence of PtO_2, H_2O. One should be able to prepare similarly the tritium-labelled hormone, but the radiochemical yield

from a limited amount of isotope would probably be low, since the introduction of isotope is made at the beginning of a long series of chemical transformations and also because isotopic labelling of the solvent employed in the catalytic reduction is required. This investigator also considered introducing the isotope into ring *C* of an appropriate unsaturated C_{21} steroid. Accordingly, pregn-9-ene-3:20-dione was prepared from 11α-hydroxyprogesterone (Peterson & Murray, 1952) according to procedures described by Mancera, Ringold, Djerassi, Rosenkranz & Sondheimer (1953) and Rosenkranz, Mancera & Sondheimer (1954), but the isolated 9:11 double bond resisted hydrogenation with *cyclo*hexane as solvent and palladium–charcoal as catalyst. Further experimentation along these lines was halted in favour of work on preparing [16-^{3}H]progesterone as described above. Another approach, requiring isotope-exchange procedure, had also been considered, but this was not promising since Fukushima & Gallagher (1952) could thereby introduce very little stably bound deuterium into the progesterone molecule, although they obtained better results with certain other C_{21} steroids: the amounts of stably bound deuterium or tritium incorporated were small, but still useful for metabolism experiments. It might be pointed out that exchange procedures are inherently inefficient in the utilization of isotope (although the degree of incorporation may be very high indeed), which is not as great an economic consideration in the case of deuterium as it is with tritium.

The potential usefulness of hydrogen isotope as auxiliary tracers for carbon and their limitations must be considered in each particular case (an excellent discussion is given by Kamen, 1951). In the author's experience (unpublished experiments) [16-^{3}H]progesterone has proved very useful in studying the intermediary metabolism of the hormone, not involving loss of the C-17 side chain; the isotope would probably be lost in that event.

SUMMARY

A comparatively simple and inexpensive route for the preparation of [16-^{3}H]progesterone of high specific activity (0·5 c/m-mole) is described.

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The Synthesis of Serum Albumin and Tissue Proteins in Slices of Rat Liver and Liver Tumour

By P. N. CAMPBELL and NANCY E. STONE

Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W. 1

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There have been many attempts in the past to compare the way in which proteins are synthesized in normal and tumour tissue. However, for the most part the comparison has been made between the synthesis of the complex mixtures of proteins contained in the two types of tissue. Zamecnik (1952) has pointed out in his review how limited is the value of such comparisons. If the biosynthesis of proteins in tumour tissue does in fact differ from that in normal tissue, then such differences are more likely to be brought to light by comparing the synthesis of a particular protein in the two types of