

[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

## Synthesis of Radioactive Cortisone<sup>1</sup>

BY DAVID K. FUKUSHIMA, THEODORE H. KRITCHEVSKY, MAXWELL LEIGH EIDINOFF AND T. F. GALLAGHER

The synthesis of radioactive cortisone has been described. The platinum catalyzed exchange of tritium for hydrogen was accomplished with 3 $\alpha$ -acetoxypregnane-3,20-dione and cortisone acetate was prepared from this compound by the method described in the preceding paper. The distribution of the isotope in the molecule was studied and the majority of the isotope was localized at C-16.

In order to study many problems dealing with the localization, transformation and ultimate fate of the adrenocortical hormones, it is necessary to have the hormones suitably labelled with a stable or radioactive isotope. Earlier studies from this Laboratory have dealt with the preparation of deuterium steroid hormones. To broaden the investigations and to enable more detailed study of adrenal hormone physiology, we have prepared cortisone with tritium in the molecule.

The starting material for the synthesis of radioactive cortisone was 3 $\alpha$ -acetoxypregnane-11,20-dione (I). The introduction of the isotope was achieved by the platinum catalyzed exchange reaction in acetic acid containing water enriched with tritium. This procedure, used for the preparation of deuterium cholesterol by Bloch and Rittenberg,<sup>2</sup> is a general reaction that has been studied for some time in our Laboratory. As will be evidenced in this report and in later work, the procedure does not give a random distribution of the isotope but on the contrary, a specific localization at one or more positions. After the exchange reaction the radioactive 3 $\alpha$ -acetoxypregnane-11,20-dione was diluted 3-fold with non-isotopic product and was refluxed in the presence of alkali in order to remove all isotope from the labile positions alpha to the carbonyl groups. It was presumed that during this reaction all, or very nearly all, of the isotope incorporated on carbons 9, 12, 17 and 21 was replaced by ordinary hydrogen. This assumption must be made with certain reservations. It is highly probable that a ketone group at C-11 enolizes to both C-9 and C-12 as evidenced by the formation of esters<sup>3</sup> with the double bond between C-9 and C-11 while bromination<sup>4</sup> of an 11-ketone results in the substitution of halogen on C-12. It is highly probable, therefore, that any isotope introduced at 9 or 12 during the exchange reaction would be replaced by the hydrogen of the medium during refluxing with alkali. The ketone on C-20, on the other hand, enolizes principally toward C-17 since bromination of the ketone yields first a 17-bromo derivative and since enol derivatives have the unsaturated bond extending from C-17 to C-20. We do not know the extent of enolization to C-21 and as will be shown in subsequent sections, removal of tritium from this terminal methyl group was not complete after two hours heating with aqueous alkali.

(1) This investigation was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the Lillia Babbitt Hyde Foundation, the Teagle Foundation, the National Cancer Institute, United States Public Health Service and the Anna Fuller Fund.

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

(3) T. H. Kritchevsky, D. L. Garmaise, T. F. Gallagher, *THIS JOURNAL*, **74**, 483 (1952).

(4) R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKensie and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

The radioactive 3 $\alpha$ -hydroxypregnane-11,20-dione was converted to 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione (IIa) and this product was rigorously purified. The radioactivity was 1410  $\mu\text{c.}/\text{mM.}$ <sup>4a</sup> The remaining stages of the synthesis of cortisone were carried out by the procedure described in the preceding paper.<sup>3</sup> Both the free alcohol IIIa and the 21-monoacetate IIIb were prepared. The products were highly radioactive, 1030  $\mu\text{c.}/\text{mM.}$  and the physical constants demonstrated that the compounds were as pure as the best products described.

Since the radioactive cortisone is intended for use in biological and biochemical experiments, it is important to determine the position occupied by the isotope. This problem is also interesting from a purely chemical standpoint since the position of the isotope is determined by the nature of the catalyst-substance complex formed during the exchange reaction in dilute acetic acid at elevated temperature. Degradation studies were undertaken, therefore, to establish the location of the isotope and advantage was made of the fact that hydrogens  $\alpha$  to a ketone group are "labile" and are replaced by hydrogen of the medium when refluxed with alkali. A hydrogen atom attached to an isolated double bond is stable under these conditions whereas hydrogen attached to the double bond of an  $\alpha,\beta$ -unsaturated ketone system is labile and exchanges with the medium. Oxidation by chromic acid under mild conditions is accomplished without generalized isotopic loss, except at the positions oxidized. Thus the oxidation of a secondary alcohol to a ketone group is accompanied by loss of isotope from the carbon bound hydrogen of the carbinol group, while isotope alpha to the resultant ketone does not exchange with the medium provided, of course, that the ketone is not heated with either acid or base. These facts are well illustrated in this study and have been amply confirmed by a number of similar investigations carried out in this Laboratory.

It was first shown that a small amount of radioactivity was still present at C-21 of both the final product cortisone and the initial material 3 $\alpha$ -hydroxypregnane-11,20-dione. When 3 $\alpha$ -acetoxy-17 $\alpha$ -hydroxypregnane-11,20-dione (IIb), 1410  $\mu\text{c.}/\text{mM.}$ , from which any possible isotope at C-17 had been eliminated during the oxidation with perbenzoic acid, was oxidized with chromic acid, 3 $\alpha$ -acetoxyetiocolane-11,17-dione (Va), 1330  $\mu\text{c.}/\text{mM.}$  was obtained. The transformation is equivalent to the removal of the C-21 methyl group and, therefore, the decrease in specific activity is equal to the amount of isotope which was initially attached to the C-21 methyl group, that is, 80  $\mu\text{c.}/\text{mM.}$  A similar result was obtained when cortisone acetate (1030  $\mu\text{c.}/\text{mM.}$ ) was oxidized to adrenosterone

(4a) Microcuries per millimole; see experimental section.

IV (910  $\mu\text{c./mM.}$ ). The loss of radioactivity (120  $\mu\text{c./mM.}$ ) is comparable although slightly larger than indicated from the preceding result. The minor discrepancy is readily understandable, since the cortisone acetate was subjected to a mild alkaline hydrolysis in aqueous medium prior to oxidation to adrenosterone. As will be clear from subsequent discussion, a certain amount of isotope in the cortisone acetate was in a "labile" position and, therefore, a slightly greater loss in this instance was expected. The value obtained for the radioactivity at C-21 from oxidation of 3 $\alpha$ -acetoxy-17 $\alpha$ -hydroxypregnane-11,20-dione is probably more nearly the true value.

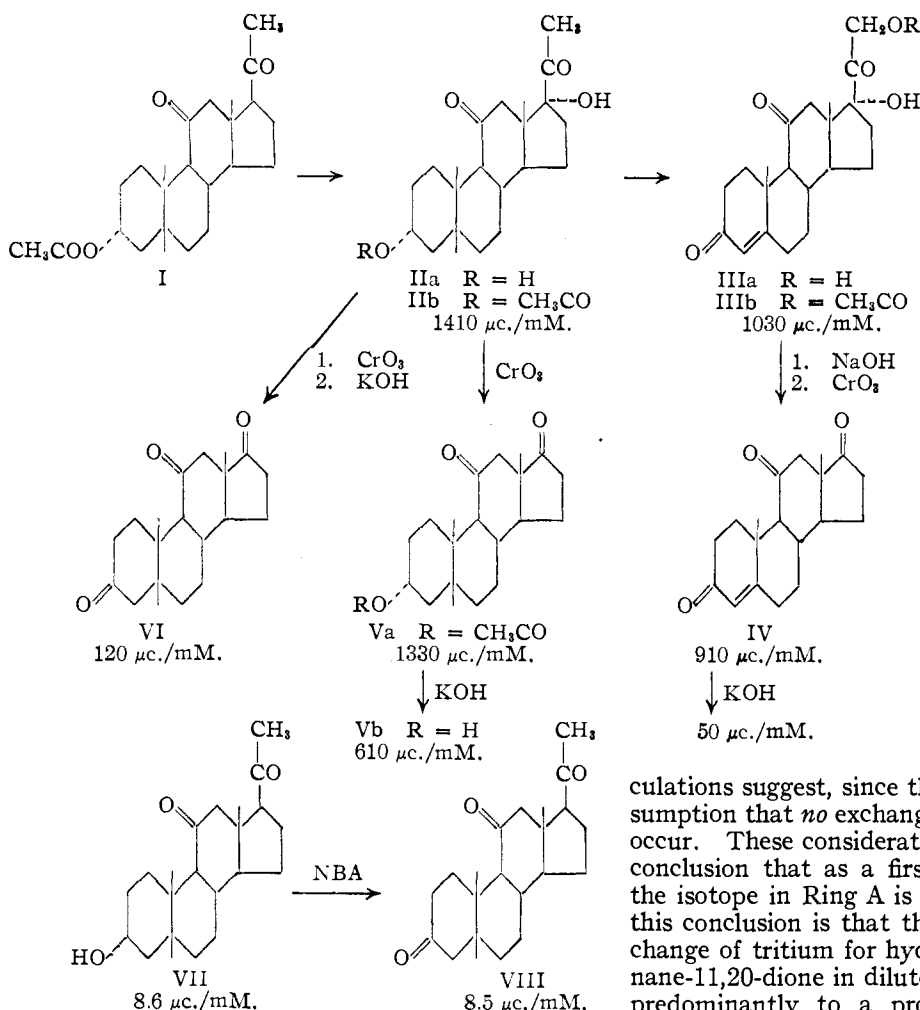
When adrenosterone (910  $\mu\text{c./mM.}$ ) was equilibrated by heating for four hours with alkali, the adrenosterone recovered had a specific activity of 50  $\mu\text{c./mM.}$  The very considerable loss of radioactivity proved that practically all of the isotope was in "labile" positions in the adrenosterone molecule. Since prior treatment had removed all of the isotope from C-9, C-12 and C-17 and since as will be demonstrated later there was no isotope at 3, the result with adrenosterone proves that most of the isotope in the cortisone nucleus must have occupied positions 2, 4, 6 or 16. The results provide strong evidence that oxidation with chromic acid does not remove any appreciable amount of isotope from the steroidal nucleus *despite the fact that practically all the isotope present was in "labile" positions.*

The distribution of tritium in the steroid nucleus was established by the following results. Oxidation of 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione (IIa) (1410  $\mu\text{c./mM.}$ ) with chromic acid followed by equilibration of the product with aqueous alkali for 2 hours, yielded etiocholane-3,11,17-trione (VI) with a specific activity of 120  $\mu\text{c./mM.}$  The loss of specific activity must be corrected for the amount previously found present on C-21, that is 80  $\mu\text{c./mM.}$ ; the difference, therefore, 1210  $\mu\text{c./mM.}$ , must have been lost from the nuclear positions 2, 4 and 16 since these are the only ones in this molecule that could have contained "labile" isotope. A very considerable amount of the 1210  $\mu\text{c./mM.}$  was at C-16. 3 $\alpha$ -Acetoxyetiocholane-11,17-dione (Va), 1330  $\mu\text{c./mM.}$ , obtained by oxidation of 3 $\alpha$ -acetoxy-17 $\alpha$ -hydroxypregnane-11,20-dione was hydrolyzed and equilibrated with aqueous alkali for two hours and yielded 3 $\alpha$ -hydroxyetiocholane-11,17-dione (Vb) with a specific activity of 610  $\mu\text{c./mM.}$  The loss, 720  $\mu\text{c./mM.}$ , represents the radioactivity which must have been present at C-16 since this is the only position in the molecule that could have contained "labile" isotope. The difference then between this value and the amount present at C-2, C-4 and C-16 (1210  $\mu\text{c./mM.}$ ) is 490  $\mu\text{c./mM.}$  and this must represent the total activity present at C-2 and C-4 together. The amount of tritium at C-5 and C-6 can be established as follows. Comparison of etiocholane-3,11,17-trione and adrenosterone ( $\Delta^4$ -androstene-3,11,17-trione) shows that these two compounds differ in terms of labile hydrogen by the hydrogen attached to C-5 and C-6. The difference in the radioactivity of the two products, 70  $\mu\text{c./mM.}$  is, therefore, a clear measure of the isotope which occupied C-5 or C-6.

The experimental results thus far have permitted us to draw the following conclusions about the distribution of radioactivity in the 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione that was used for the synthesis of cortisone: C-16 contained 720  $\mu\text{c./mM.}$ ; C-2 and C-4 together contained 490  $\mu\text{c./mM.}$ ; C-21 contained 80  $\mu\text{c./mM.}$ ; and C-5 and C-6 combined contained 70  $\mu\text{c./mM.}$ ; stably bound and location undetermined 50  $\mu\text{c./mM.}$  The sum of the specific activities at these carbons accounts for all of the activity in the equilibrated 3 $\alpha$ -hydroxypregnane-11,20-dione from the exchange reaction.

It would be desirable to draw a conclusion about the amount of isotope at C-4 in both 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione as well as cortisone acetate and, with reservations, this is possible. When 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione was transformed to cortisone acetate there was a loss of 380  $\mu\text{c./mM.}$  The preceding evidence has shown 80  $\mu\text{c./mM.}$  in the C-21 methyl group. In the preparation of cortisone, one of the hydrogens of the methyl group was replaced with an alcohol group and thus one-third of the activity or 25  $\mu\text{c./mM.}$  should have been lost from the side chain. In a preliminary experiment a diluted sample of the radioactive 3 $\alpha$ -hydroxypregnane-11,20-dione (VII), 8.6  $\mu\text{c./mM.}$ , was oxidized with N-bromoacetamide and the pregnane-3,11,20-trione (VIII) isolated contained 8.5  $\mu\text{c./mM.}$  It is apparent, then, that within experimental error, there was no isotope at C-3. Therefore if the transformation of 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione to cortisone acetate were accomplished without any loss of isotope save the 25  $\mu\text{c./mM.}$  from C-21, the final product would be expected to have 1385  $\mu\text{c./mM.}$  while as an experimental fact it had a specific activity of 1030  $\mu\text{c./mM.}$  Since this difference (355  $\mu\text{c./mM.}$ ) must certainly have been from hydrogens on C-4 and 5 and possibly from hydrogens attached to C-2 or C-6, an estimate can be made of the radioactivity that each of these four sites contributed to the loss.

Let it be assumed that formation of the  $\alpha,\beta$ -unsaturated ketone system in Ring A was effected without preferential reaction of hydrogen or tritium atoms and that there was no equilibration of "labile" hydrogen. Although the rate and extent of enolization of the intermediates are very important in these considerations, the latter assumption is obligatory in order to arrive at a conclusion that is in reasonable agreement with experimental fact. A very strong argument can be adduced for C-2 on the basis of these conditions. If the specific activity thus far referred to as located at C-2 and C-4 together (490  $\mu\text{c./mM.}$ ) were actually all at C-2 the preparation of cortisone acetate should have been achieved without any serious loss of isotope. This would follow from the fact that 3-ketones of the normal series brominate predominantly at C-4. Thus, no isotope would be lost in the bromination step and the only possibility for loss of isotope from C-2 would result from enolization of the 4-bromo-3-ketone toward C-2 or exchange of the isotope in subsequent stages of the formation of the  $\alpha,\beta$ -unsaturated ketone. It is little likely that 4-bromo-3-ketones of the cholane series would enolize extensively toward C-2 especially at low temperature,



made of the 70  $\mu\text{c./mM.}$ , located at C-5 and C-6 and the not unreasonable hypothesis made that all of this has been lost either by direct abstraction in the dehydrobromination or by exchange with the medium from C-6 during cleavage of the semicarbazone, then the calculated loss of 315  $\mu\text{c./mM.}$  closely approximates the experimentally determined figure. Moreover, since the formation and cleavage of the semicarbazone in acetic-pyruvic acid was carried out at 65–70°, it is quite likely that there was some replacement of the "labile" hydrogen at C-4. Therefore, a greater loss would be expected than the cal-

culations suggest, since these were based on the assumption that no exchange with the medium would occur. These considerations therefore, lead to the conclusion that as a first approximation most of the isotope in Ring A is at C-4. The corollary of this conclusion is that the platinum catalyzed exchange of tritium for hydrogen of 3 $\alpha$ -acetoxypregnane-11,20-dione in dilute acetic acid at 150° leads predominantly to a product stably isotopically labeled at C-4 and C-16.

From these deductions it is then possible to arrive at a good estimate of the isotopic distribution in the cortisone acetate obtained in this investigation. The specific activity of the product, 1030  $\mu\text{c./mM.}$ , is distributed as follows: 720  $\mu\text{c./mM.}$  or 70% is at C-16, 50  $\mu\text{c./mM.}$  or 5% is at C-21, 50  $\mu\text{c./mM.}$  or 5% is stably bound and not located in the present investigation and 210  $\mu\text{c./mM.}$  or 20% is at C-4. It should be emphasized that the specific activity of C-4 is a maximum value and the actual amount at this position may be less than this figure.

### Experimental<sup>5</sup>

**Measurement of Radioactivity.**—The steroids were burned in a stream of oxygen using a platinum filling in the combustion tube. The water was trapped at Dry Ice temperature and was converted to hydrogen gas by passage through a zinc filled tube at 400° in the absence of air. The hydrogen gas was introduced into an 18-mm. diameter counter tube having a silvered cathode and a 2-mil tungsten anode wire. The counting gas mixture consisted of approx. 5-cm. pressure of hydrogen and 65-cm. pressure of methane. Measurements were made 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

in acetic acid in the presence of sufficient sodium acetate to buffer most of the hydrobromic acid formed in the substitution reaction. The  $\Delta^4$ -3-ketone system of cortisone enolizes almost exclusively toward C-6. In both events little isotope would be lost from C-2 while in actuality a very considerable amount of isotope was lost in the transformation of 3 $\alpha$ ,17 $\alpha$ -dihydroxypregnane-11,20-dione (IIa) to cortisone acetate (IIIb). The assumption, therefore, that all of the isotope was at C-2 obviously does not fit the facts.

If it be assumed that the radioactivity thus far referred to as at C-2 and C-4 together were equally distributed among the 4 hydrogen atoms at both C-2 and C-4, the loss of at least one-quarter the total, or 120  $\mu\text{c./mM.}$ , might be expected in the formation of cortisone. Even if all of the isotope at C-4 had been lost through bromination and exchange, the total activity lost should have been 245  $\mu\text{c./mM.}$  instead of 355  $\mu\text{c./mM.}$  experimentally determined. While the possibility that the isotope was equally distributed between C-2 and C-4 has not been rigidly excluded, it is unlikely. This leaves as a last alternative the presumption that all or most of the tritium was on C-4. If this were so and the isotope was equally distributed between equatorial and polar hydrogens, one-half or 245  $\mu\text{c./mM.}$  should have been lost, a value in fair agreement with the actual loss of 355  $\mu\text{c./mM.}$  When consideration is

(5) All melting points were taken in capillary tubes and are corrected. The phrase "in the usual way" means the organic solvent was washed with either acid or base or both, as appropriate, followed by washing with water and drying the solution over sodium sulfate before distillation of the solvent.

greater than one millivolt. Under these conditions, the counting plateau extends for approximately 800 volts with a slope of less than 0.3% per hundred volts. The sensitive volume of the counter tube was measured in the cylindrical region defined by the glass shield extremities at each end of the anode wire.

The activity of the hydrogen gas in terms of disintegrations per millimole of hydrogen was determined using the sensitive volume and the measured pressure and temperature of the filling. Taking  $2.22 \times 10^8$  disintegrations per minute equal to one microcurie, the activity in microcuries per millimole of steroid compound is given by the expression  $\mu\text{c./mM.} = \frac{1}{4.44 \times 10^8} \left( \frac{CRTA}{PV} \right)$  where (C) is the measured counting rate (c.p.m.) for the counter tube with sensitive volume (V) in ml. filled at hydrogen pressure (P) in atm. at T°K. (R) is the gas constant, 82.05 ml. atm./deg. mole, and A is the number of hydrogen atoms per molecule of steroid. Sufficient counts were taken to reduce the statistical error to less than one per cent.

Triplicate analyses were made in several instances. It was later found more convenient to discard the hydrogen gas from the initial combustion and conversion and to determine the activity of the second and third combustions and conversions. In all cases these duplicate analyses checked within 2% so that memory in the train and counting tube was negligible.

The absolute activities may be in error by 5–10% as a result of inhomogeneity of the electric field at the counter ends.

**3 $\alpha$ ,17 $\alpha$ -Dihydroxy-*t*-pregnane-11,20-dione (IIa).**—A suspension of 250 mg. of Adams catalyst in 70% acetic acid and tritium enriched water was pre-reduced with tritium enriched hydrogen gas. To the mixture were added 500 mg. of 3 $\alpha$ -acetoxy-pregnane-11,20-dione and 9 ml. of tritium enriched 70% acetic acid. The reaction flask was frozen, evacuated and sealed. It was then rotated at 150° for two days. The catalyst was removed by filtration and the solvent recovered by distillation *in vacuo* in a closed system. Two more runs were made in the same manner, each time with the use of the recovered solvent. The residues from the three runs were combined and refluxed for 2 hours under nitrogen with 75 ml. of methanol, 15 ml. of water and 60 ml. of 5% methanolic potassium hydroxide. The reaction mixture was worked up in the usual way and 800 mg. of non-isotopic 3 $\alpha$ -hydroxypregnane-11,20-dione was added. The diluted mixture was chromatographed on silica gel. Elution with acetone-ether mixtures (0.3% to 20%) yielded 1.72 g. of crude 3 $\alpha$ -hydroxy-*t*-pregnane-11,20-dione. This radioactive steroid was converted without purification to IIa by the procedure described in the preceding paper<sup>3</sup>; 1.80 g. of crystalline product was obtained. The analytical sample of 3 $\alpha$ ,17 $\alpha$ -dihydroxy-pregnane-11,20-dione melted at 200–202°, unchanged upon admixture with an authentic sample; 1410  $\mu\text{c./mM.}$

***t*-Cortisone Acetate (IIIb).**—3 $\alpha$ ,17 $\alpha$ -Dihydroxy-*t*-pregnane-11,20-dione was converted into cortisone acetate following the method described in the preceding paper.<sup>3</sup> The product had the following physical characteristics—m.p. 245–246°;  $[\alpha]_D^{20} +186^\circ$  (acetone),  $\epsilon_{2380}$  16,000 (95% ethanol); 1030  $\mu\text{c./mM.}$  The 6-dehydro derivative could not be detected from the ultraviolet spectrum. The free alcohol IIIa melted at 226–228° (evacuated capillary)  $\epsilon_{2380}$  15,400. The infrared spectra of both acetate and free alcohol were indistinguishable from authentic pure specimens.

***t*-Adrenosterone (IV).**—A stream of nitrogen was bubbled through a solution of 2.00 g. of *t*-cortisone acetate (0.452  $\mu\text{c./mM.}$ ; dilution factor 2285) in 1 l. of 95% ethanol for one half hour. One liter of 0.1 *N* sodium hydroxide solution was then added with stirring and the solution was allowed to stand for 40 min. in a nitrogen atmosphere. The hydrolysis mixture was diluted with 10% sodium chloride and extracted with ethyl acetate. The ethyl acetate extract was dried over sodium sulfate and the solvent was removed *in vacuo*. The residue was dissolved in 75 ml. of glacial acetic acid and to it was added 75 ml. of 1% chromium trioxide in glacial acetic acid solution. The oxidation mixture was allowed to stand for 17 hours at room temperature and the product was worked up in the usual

way. Recrystallizations from methanol gave adrenosterone, m.p. 222–223°,  $\epsilon_{2380}$  15,500 (95% ethanol), 0.39  $\mu\text{c./mM.}$ , corresponding to 910  $\mu\text{c./mM.}$  in the undiluted product. The infrared spectrum of IV was identical with that of an authentic sample.

A solution of 200 mg. of IV in 50 ml. of methanol, 40 ml. of 5% methanolic potassium hydroxide and 10 ml. of water was refluxed for 4 hours under nitrogen. After purification by chromatography, sublimation and recrystallization, the adrenosterone had an activity of 0.222  $\mu\text{c./mM.}$  corresponding to 50  $\mu\text{c./mM.}$  in the undiluted product.

***t*-Etiocolane-3,11,17-trione (VI).**—To a solution of 900 mg. of 3 $\alpha$ ,17 $\alpha$ -dihydroxy-*t*-pregnane-11,20-dione (0.628  $\mu\text{c./mM.}$ ; dilution factor 2245) in 25 ml. of acetic acid was added a solution of 1.9 g. of chromium trioxide in 65 ml. of 90% acetic acid. The oxidation mixture was allowed to stand for one hour and was worked up in the usual way to give 220 mg. of oil. The oily residue was refluxed in 10 ml. of methanol, 3 ml. of water and 10 ml. of 5% methanolic potassium hydroxide for two hours. The base equilibrated product was purified by chromatography on alumina and recrystallization from ether-petroleum ether to give etiocolane-3,11,17-trione, m.p. 130–131°, no depression of the m.p. when admixed with an authentic sample; 0.0536  $\mu\text{c./mM.}$  corresponding to 120  $\mu\text{c./mM.}$  in the undiluted product. The infrared spectrum was identical with that of an authentic sample.

**3 $\alpha$ -Acetoxyetiocolane-11,17-dione (Va).**—To a solution of 850 mg. of 3 $\alpha$ -acetoxy-17 $\alpha$ -hydroxy-*t*-pregnane-11,20-dione (0.611  $\mu\text{c./mM.}$ ; dilution factor 2305) in 20 ml. of acetic acid was added 1.6 g. of chromium trioxide in 60 ml. of 90% acetic acid. The oxidation mixture was allowed to stand for 1.5 hours and was worked up in the usual way. Recrystallizations from methanol and ethyl acetate-petroleum ether gave 3 $\beta$ -acetoxy-*t*-etiocolane-11,17-dione, m.p. 159–161°; no depression when admixed with an authentic sample, 0.576  $\mu\text{c./mM.}$  corresponding to 1330  $\mu\text{c./mM.}$  in the undiluted product. The infrared spectrum was identical with that of an authentic sample of Va.

**3 $\alpha$ -Hydroxyetiocolane-11,17-dione (Vb).**—A solution of 350 mg. of 3 $\alpha$ -acetoxy-*t*-etiocolane-11,17-dione was refluxed for 2 hours with 10 ml. of methanol, 3 ml. of water and 10 ml. of 5% methanolic potassium hydroxide. Purification by chromatography on alumina and recrystallization from acetone-petroleum ether gave 3 $\alpha$ -hydroxy-*t*-etiocolane-11,17-dione, m.p. 187–188.5°, no depression of the m.p. when admixed with an authentic sample; 0.265  $\mu\text{c./mM.}$  corresponding to 610  $\mu\text{c./mM.}$  in the undiluted product. The infrared spectrum was identical with that of an authentic sample of Vb.

**Oxidation of 3 $\alpha$ -Hydroxy-*t*-pregnane-11,20-dione (VII).**—A small sample of 3 $\alpha$ -hydroxy-*t*-pregnane-11,20-dione was diluted with 1.2 g. of non-isotopic 3 $\alpha$ -acetoxy-pregnane-11,20-dione and saponified in 100 ml. of 0.3 *N* sodium hydroxide in 50% ethanol for 30 minutes. The product, a mixture of 3 $\alpha$ -hydroxy-*t*-pregnane-11,20-dione and the 17 $\alpha$ -epimer, was recrystallized twice from acetone and melted at 161–165°; 8.6  $\mu\text{c./mM.}$  To a solution of 1.07 g. of diluted 3 $\alpha$ -hydroxy-*t*-pregnane-11,20-dione in 25 ml. of *t*-butanol and 1.5 ml. of water was added 1.1 g. of *N*-bromoacetamide. The solution was stored in the refrigerator for 18 hours and was worked up in the usual way. Two recrystallizations from petroleum ether-acetone gave diluted *t*-pregnane-3,11,20-trione, m.p. 151–154°; 8.5  $\mu\text{c./mM.}$  The m.p. indicates a mixture with the 17 $\alpha$ -epimer.

**Acknowledgments.**—We wish to express our appreciation to Robert W. Jailer and Joseph Knoll who carried out the measurements of radioactivity. The tritium was supplied by the Isotopes Division of the Atomic Energy Commission. We are greatly indebted to Dr. Max Tishler, Merck and Co., Inc., who furnished us with generous supplies of 3 $\alpha$ -acetoxy-pregnane-11,20-dione and cortisone acetate. Dr. Konrad Dobriner of this Institute determined and interpreted the infrared spectra.

NEW YORK 21, N. Y.

RECEIVED JULY 16, 1951