removed and the residue was recrystallized from aqueous methanol to give 250 mg (34%) of the ester, mp 114–116°; $[\alpha]$D 95.6° (c 2.8 MeOH) (lit. mp 110–111°; $[\alpha]$D 96.5°);[21] ir (KBr) 1735, 1700 cm^{-1}; R_f 0–0.1; nmr δ 1.02 (18- and 19-CH_3), 2.29 (broad, 3β-H); ORD (c 1.12, MeOH) $[\Phi]_{400}$ 133°, $[\Phi]_{350}$ 223°, $[\Phi]_{304}$ 580°, $[\Phi]_{255}$ 223°, $[\Phi]_{230}$ 534°.

Methyl 3-Hydroxy-4-keto-12α-acetoxy-5α-chol-2-enate (16).—To a solution of 447 mg (1.0 mmol) of methyl 3-keto-12α-acetoxy cholanate[22] in 100 ml of *t*-butyl alcohol (freshly distilled from calcium hydride) was added a solution of 1.12 g (10.0 mmol) of potassium *t*-butoxide in 80 ml of *t*-butyl alcohol. The resulting solution was stirred under 1 atm of oxygen until 11.2 ml (1.0 mmol) had been taken up, then it was acidified with hydrochloric acid, diluted with water, and extracted with ether. The extract was washed with water, dried ($MgSO_4$), evaporated, and the residue was esterified by boiling under reflux for 3 hr in a 15% solution of methanolic HCl. After removal of the solvent, the residue was taken up in benzene and chromatographed on 20 g of silica gel. Elution with 5% ether–benzene gave the diosphenol **16** which was recrystallized from aqueous methanol to give 100 mg (22%), mp 160–162°; $[\alpha]$D 111° (c 3.9, MeOH); ir (KBr) 1730, 1725, 1660, 1625 cm^{-1}; R_f 0.45; uv λ_{max} (MeOH) 277.9 mμ (ϵ 24,000); nmr δ 1.96 (12-$OCOCH_3$), 5.08 (12β-H), 6.00 (t, 2-H).

Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.41; H, 8.75. Found: C, 69.75; H, 8.78.

Methyl 3α-Carbomethoxy-3β,12α-dihydroxy-A-nor-5α-cholanate (17).—To a solution of 1.95 g (4.2 mmol) of the diosphenol **16** in 100 ml of *n*-butyl alcohol was added a solution of 14 g of KOH and 10 ml of water, and the resulting solution was boiled under reflux for 3 days. After acidification with hydrochloric acid and dilution with water, the reaction mixture was extracted with ether and the extract was washed with water, dried ($MgSO_4$), and evaporated. The residue was esterified by boiling under reflux for 3 hr with 15% methanolic HCl, the solvent was evaporated, and the residue was taken up in benzene and chromatographed on silica gel. Benzene eluted an oil which was crystallized from aqueous methanol to give 178 mg (9.4%) of the A-norhydroxy ester **17**, mp 53–54°; $[\alpha]$D 36° (c 1.7, MeOH); ir (KBr) 1730, 1720, 1018–1036 cm^{-1}; nmr δ 0.98 (19-CH_3), 3.58, 3.68 ($OCOCH_3$), 3.95 (12β-H).

Anal. Calcd for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40. Found: C, 70.33; H, 9.51.

Methyl 3-Keto-12α-hydroxy-A-norcholanate (15a).—The diester **17**, 86 mg, was first hydrolyzed to the dihydroxy diacid in quantitative yield by warming in a solution of methanolic potassium hydroxide. Acidification with hydrochloric acid and dilution with water gave the crystalline diacid, mp 96–98°, which was dissolved in 5 ml of acetic acid and 2 ml of acetic anhydride. Lead oxide (Pb_3O_4), 287 mg, was added and the mixture was warmed on a steam bath until the red color disappeared, stirred overnight, diluted with water, and extracted with ether. The extract was washed with water, several times with 10% $NaHCO_3$ solution, and water, dried ($MgSO_4$), and evaporated. The residue was esterified by boiling under reflux for 3 hr with 15% methanolic HCl. Removal of the solvent and recrystallization of the residue gave 30 mg of the 3-keto-A-nor ester **15a**, identical in melting point and ir spectrum with the material described above.

The oxidation did not take place if the diester was used instead of the hydrolyzed material.

Registry No.—**2**, 20414-15-7; **3**, 20414-16-8; **6**, 20445-42-5; **7a**, 20414-17-9; **7b**, 20414-18-0; **7c**, 20414-19-1; **7d**, 20414-20-4; **8a**, 20414-21-5; **8b**, 20414-22-6; **8c**, 20414-23-7; **8d**, 20414-24-8; **9**, 20445-43-6; **10**, 20445-44-7; **13a**, 20414-25-9; **13b**, 20414-26-0; **14a**, 20414-27-1; **15a**, 20414-28-2; **16**, 20414-29-3; **17**, 20414-30-6.

(21) T. Reichstein and M. Sorkin, *Helv. Chim. Acta*, **25**, 797 (1942).
(22) T. Reichstein and V. Burchhardt, *ibid.*, **25**, 821 (1942).

Further Stereochemical Studies of the Catalytic Reduction of $\Delta^{1,4}$-3-Keto Steroids with Tritium[1a]

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Received December 4, 1968

The stereochemical distribution of label resulting from reduction of $\Delta^{1,4}$-steroids at C-1,2 with tritium gas using palladium–charcoal was studied. The tritium distribution at C-1 in 11β-hydroxyandrost-4-ene-3,17-dione and 11β,17,21-trihydroxypregn-4-ene-3,20-dione (cortisol) obtained from the corresponding Δ^1 compounds was analyzed using stereospecific chemical and enzymatic reactions. The distribution was found to be 76% β and 24% α. This is in general agreement with results obtained previously after reduction of a compound without an 11β-hydroxyl group. Analysis of testosterone-1,2-*t* using the C-1,2-dehydrogenase of *B. sphaericus* and the ring A aromatase (estrogen forming) enzyme system from human placenta indicated that the tritium distribution at C-2 was in a ratio of 1.4:1 (β:α), considerably less than that at C-1, 3.4:1 (β:α). A mechanism of reduction involving 1,4 addition to the enone system is discussed. The results are in agreement with our previous finding that estrogen formation in placenta involves *cis* elimination at C-1,2 (1β,2β).

Catalytic reduction of carbon–carbon double bonds with carrier-free tritium gas is a facile method for preparing pure radioactive compounds of high specific activity at relatively low cost. However, the distribution and orientation of tritium in the product is often not apparent. Owing to the instability of highly tritiated molecules and the dangers of contamination, physical measurements used for deuterated compounds are not made routinely on tritiated species. Instead, stereospecific reactions with diluted material and extrapolation of results obtained with deuterium often are used to determine the position labeled.[2] In previous publications, methods were discussed which enabled us to determine the distribution of tritium at positions 1,[3] 6, 7,[4,5] 11, and 12[6,7] of the steroid nucleus. The study of

(1) (a) This work was supported in part by U. S. Public Health Service Grants AM-6894, AM-3419, and Training Grant T01-AM05564. An abstract of portions of this work has appeared: H. J. Brodie, K. Raab, and M. Gut, *Excerpta Med. Int. Congr. Ser., 111, Int. Congr. Hormonal Steroids, 2nd, Milan, 1966*, Abstract No. 178. (b) McGill University Cancer Research Unit, Montreal. (c) Postdoctoral trainee in the Training Program for Steroid Biochemistry, 1963–1965; Pharma Research Canada, Pointe Claire, Quebec. (d) University of Tokyo, Tokyo, Japan.

(2) E. A. Evans, "Tritium and Its Compounds," Van Nostrand, New York, N. Y., 1966.
(3) H. J. Brodie, M. Hayano, and M. Gut, *J. Amer. Chem. Soc.*, **84**, 3766 (1962).
(4) S. Baba, H. J. Brodie, M. Hayano, G. Kwass, and M. Gut, *J. Org. Chem.*, **29**, 2751 (1964).
(5) H. J. Brodie, S. Baba, M. Gut, and M. Hayano, *Steroids*, **6**, 659 (1965).
(6) M. Hayano, M. Gut, R. I. Dorfman, O. K. Sebek, and D. H. Peterson, *J. Amer. Chem. Soc.*, **80**, 2336 (1958).
(7) M. Hayano, M. Gut, R. I. Dorfman, A. Schubert, and R. Siebert, *Biochim. Biophys. Acta*, **32**, 269 (1959).

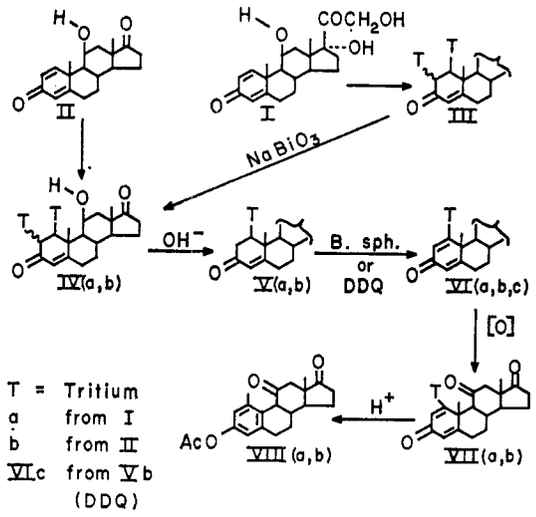
tritium introduction at C-1 initially was carried out in order to obtain estrogen precursors suitable for stereochemical studies, and we communicated that 17β-hydroxyandrosta-1,4-dien-3-one was reduced at the Δ^1-bond with palladium–carbon to give testosterone with the tritium at C-1 approximately 80% β, while the reduction of 5α-androst-1-ene-3,17-dione gave the saturated steroid with the label at C-1 over 90% α. The detailed methodology appeared in a subsequent paper dealing with the preparation of 19-hydroxy- and 19-nor steroids stereoselectively labeled at C-1β.[8]

Other steroids which are labeled at C-1,2 by reduction of $\Delta^{1,4}$ compounds contain the 11β-hydroxy function. They are used extensively for metabolic studies and it was of interest to determine whether the additional β substituent, 1,3 diaxial to the C-10 methyl group, has an effect on the stereochemistry of reduction of the Δ^1-bond. This paper reports on the reduction of such compounds. In addition, data are presented concerning the complete distribution of tritium in a commercial sample of testosterone-1,2-*t* prepared by similar reduction of 17β-hydroxyandrosta-1,4-dien-3-one.

Results

A. Tritium at C-1 in 11β-Hydroxy Compounds.—The two compounds chosen for reduction were prednisolone (I) (11β,17,21-trihydroxypregna-1,4-diene-3,20-dione) and 11β-hydroxyandrosta-1,4-diene-3,17-dione (II). The cortisol-1,2-*t* (III) from reduction of I with palladium–carbon catalyst[9] (Scheme I)

SCHEME I



was diluted with carrier and the side chain was cleaved with sodium bismuthate[10] to give 11β-hydroxyandrost-4-ene-3,17-dione-1,2-*t* (IVa). This and the same compound IVb obtained from the similar reduction of the Δ^1 bond of II were recrystallized to constant specific activity and then were equilibrated with base to remove enolizable tritium. After correction for stable tritium (see below), it was found that, whereas 45% of the tritium in IVb was enolizable to give Vb, only 24% was lost from IVa to give Va (Table I, line 3). Because of

(8) H. J. Brodie, *Tetrahedron*, **23**, 535 (1967).
(9) P. Osinski and A. Vanderhaeghe, *Rec. Trav. Chim.*, **79**, 216 (1960).
(10) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

TABLE I
CHANGES IN TRITIUM CONTENT AFTER INDICATED CONVERSIONS

	IV		V		VI[b]			VIII	
	a[a]	b	a	b	a	b	c	a	b
Relative *t* content	100	100	79	58	63	45	45	9.6	7.1
Relative reactive *t* content[c]	90	93	69	51	53	38	38	0	0
% "reactive" *t* lost from preceding step	...	...	24	45	23	25	25	100	100

[a] "a" compounds obtained from compound I. "b" compounds obtained from compound II. [b] Compounds a and b are from incubation with *B. sphaericus*. VIc is from DDQ reaction on Vb. [c] Relative amount of tritium in the molecule less the amount of stable tritium remaining in VIII (stable tritium). Thus Va contains 69% reactive tritium (79–9.6).

suggestions in review articles (ref 2, p 362)[11,12] that Δ^4-3 ketones form Δ^2-enols with difficulty,[13,14] a sample of 11β-hydroxyandrost-4-ene-3,17-dione was refluxed for 4 hr with potassium hydroxide in 98% deuterium-enriched methanol–water. Combustion analysis of the recovered steroid showed an incorporation of 6.5 atoms of deuterium, indicating that the seven enolizable hydrogens are exchangeable.

Compounds Va and Vb were incubated with respiring cultures of *B. sphaericus* to give the C-1,2 dehydrogenated products VIa and VIb with tritium losses from C-1 of 23 and 25% respectively (Table I, line 3). Since the C-1,2-dehydrogenase of *B. sphaericus* removes the 1α and 2β hydrogens,[15] the results show that the distribution of label at the C-1 was about 75% β and 25% α. The equilibrated material Vb from the reduction of 11β-hydroxyandrostadienedione (II) also was subjected to chemical dehydrogenation with DDQ (2,3-dichloro-5,6-dicyanoquinone), which also preferentially removes the 1α hydrogen[3,16] to give the Δ^1 analog VIc with substantially the same result (25% loss of tritium) (Table I, line 3). Jones oxidation[17] of a mixture of VIb and VIc to the 11 ketone VIIb and equilibration with base was accomplished without loss of tritium. After acid-catalyzed, dienone–phenol rearrangement to 3-acetoxy-1-methylestra-1,3,5(10)-triene-11,17-dione (VIIIb)[18] in which the C-10 methyl group migrates to displace the C-1 hydrogen,[19] 7% of the tritium originally present in 11β-hydroxyandrostenedione (IVb) remained. When Va was treated in the same manner to give VIIIa, 9.6% of the tritium originally in IVa remained (Table I, line

(11) P. Osinski, "Tritium in the Biological Sciences," Vol. II, International Atomic Energy Agency, Vienna, 1962, p 117.
(12) P. Osinski, *Atomlight*, No. 35, 6 (1964).
(13) These claims stem from a report[9] that the bismethylene dioxide of cortisol-1,2-*t* does not lose tritium on base treatment. Our results recorded here with 11β-hydroxyandrost-4-ene-3,17-dione (the large tritium loss in going from IVb to Vb and the 6.5 atoms of deuterium incorporation) and testosterone-1,2-*t* (43% *t* loss on base treatment) and our other reports[3,8] show that C-19 steroids readily exchange tritium at C-2 with base. In addition, Malhotra and Ringold[14] found that, with testosterone, base-catalyzed deuterium exchange occurs *preferentially* at the C-2β position.
(14) S. K. Malhotra and H. J. Ringold, *J. Amer. Chem. Soc.*, **86**, 1997 (1964).
(15) H. J. Ringold, M. Hayano, and V. Stefanovic, *J. Biol. Chem.*, **238**, 1960 (1963).
(16) A. B. Turner and H. J. Ringold, *J. Chem. Soc., C*, 1720 (1967).
(17) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *ibid.*, 39 (1946).
(18) E. J. Bailey, J. Elks, J. F. Oughton, and L. Stephenson, *ibid.*, 4535 (1961).
(19) E. Caspi, P. K. Grover, and Y. Shimizu, *J. Amer. Chem. Soc.*, **86**, 2463 (1964).

TABLE II
ENZYMATIC CONVERSION OF 1,2-*t*-4-^{14}C SUBSTRATES

Substrate[a]	$^3H/^{14}C$ ratio (dpm)	System	Recovered substrate		Product	
			Ratio	(% *t* change)	Ratio	(% *t* change)
A-1,2-*t*-4-^{14}C	25.3	*B. sph*, cell free	30.2	(+20)	10.8[b]	(−57)
A-1,2-*t*-4-^{14}C	23.6	*B. sph*, whole cell	24.6	(+4.2)	14.6[b]	(−38)
T-1,2-*t*-4^{14}C	26.5	Placental, supernate	26.3[c] 27.6[d]	(∼0) (+4.2)	8.3[e]	(−69)

[a] A, androst-4-ene-3,17-dione; T, testosterone. [b] Androsta-1,4-diene-3,17-dione. [c] Androst-4-ene-3,17-dione. [d] Testosterone. [e] Estrone.

1). The remaining label in VIII represents stable tritium which, from the procedures used, is at one or more of positions 7, 8, 14, 15, 18, and 19. It has been subtracted from the total tritium in the samples to give the corrected value (Table I, line 2) of tritium at C-1 and, for compound IV, C-1 and C-2.

B. Tritium at C-1,2 in Testosterone-1,2-*t*.—A purified commercial sample of testosterone-1,2-*t*, prepared by the same general method as were the 11β-hydroxy compounds above, was analyzed for tritium at C-1,2 as previously described.[3,8] After Jones oxidation to androstenedione-1,2-*t* without loss of tritium and equilibration with base, C-1,2 dehydrogenation of the resultant androstenedione-1-*t* with *B. sphaericus* established that the distribution of tritium was 44% 1β, 17% 1α, and 43% enolizable. Conversion of androstenedione-1-*t* through the $\Delta^{4,6}$ and $\Delta^{1,4,6}$ intermediates to 3-acetoxy-1-methylestra-1,3,5(10),6-tetraen-17-one showed that 1% stable tritium was present in the original testosterone-1,2-*t*.

To obtain insight into the distribution of the 43% enolizable tritium, presumably at C-2, the testosterone-1,2-*t* was aromatized to estrone with a 10,000 × g supernate preparation from human placenta. In addition, androstenedione-1,2-*t* (prepared from testosterone-1,2-*t* by Jones oxidation without significant *t* loss) was C-1,2 dehydrogenated using a cell-free preparation from *B. sphaericus* and the whole-cell respiring organism. Different per cent tritium decreases were obtained in the products, as shown in Table II. With the bacterial cell-free preparation, the apparent tritium loss on C-1,2 dehydrogenation was 57%; from the respiring culture, an apparent loss of 38% was obtained in going to the $\Delta^{1,4}$-product. With the placental system, a 69% loss of tritium was obtained on conversion to product estrone.

Discussion

Results obtained for the reduction of the two 11β-hydroxy-$\Delta^{1,4}$-3-keto compounds I and II with tritium on palladium–charcoal catalyst show that attack occurs mainly from the β side to give products 75 to 77% tritiated at C-1β. This selectivity of tritium at C-1 approximates that obtained (78 to 83% 1β) when 17β-hydroxyandrosta-1,4-dien-3-one was reduced under similar conditions as reported here and elsewhere.[3,8] Thus, the 11β-hydroxy group has little effect on the stereochemistry of the reduction process at C-1. It is not clear why there was a relatively small 24% of enolizable tritium in IVa obtained from cortisol (III) compared with approximately 44% in the reduction of 11β-hydroxyandrosta-1,4-diene-3,17-dione and 17β-hydroxyandrosta-1,4-dien-3-one. Although our preparations were not analyzed for tritium loss during C-17,20 cleavage with bismuthate, Burstein, *et al.*,[20] found no loss in this step. The smaller amount is probably not related to the presence of the additional hydroxyl hydrogens on prednisolone which by catalytic exchange with tritium may provide hydrogen for addition at C-2, since the reduction of 6β-hydroxyprednisolone gave 37% exchangeable tritium.[20] It may be noted that in the reduction of a Δ^{11} bond, 30% of the label was at C-11 and 70% was at C-12.[6]

Both reduction products contained some stable tritium (9.6 and 7.1 from I and II respectively) which was not located in ring A. This is in contrast to the commercial sample of testosterone-1,2-*t*, in which 99% of the tritium was enolizable or at C-1. Since there was no tritium lost from the 9α,11α or 12 positions during conversion of VIb to equilibrated VII or after side-chain cleavage in going from I to III,[20] it is unlikely that a significant amount of this stable tritium arises from exchange. The possibility that during the dienone–phenol rearrangement (VII to VIII) some tritium migrated from C-1 to C-2 was considered unlikely since Burstein, *et al.*,[20] using cortisol prepared from the same prednisolone, also found 8% tritium at positions other than in ring A using a procedure involving direct equilibration of tritium from the C-1 position. Since starting material II was prepared from I, it is possible that the stable tritium arose from a small amount of undetected polyene in these compounds.

Testosterone-1,2-*t* was found to have 44% 1β, 13% 1α, and 43% exchangeable tritium. These values are in good agreement with those obtained[3,8] from samples of testosterone-1,2-*t* prepared in this laboratory, also by palladium–charcoal-catalyzed reduction, and should enable the stereochemical results obtained by us with the C-1 tritiated compound[8,21–23] to be correlated with those that may be obtained with commercial material. However, it is well to recall that the same type of reduction has been achieved recently with the soluble catalyst tris(triphenylphosphine)rhodium(I) chloride, which introduces the label about 85% stereoselectively at 1α and 2α.[24,25] From the nature of the reaction, it would be expected that the exchangeable tritium is located predominantly at C-2, arising from reduction of the Δ^1 bond. This is supported by our previous experience with testosterone-1,2-*t*[3,8] and 3β-hy-

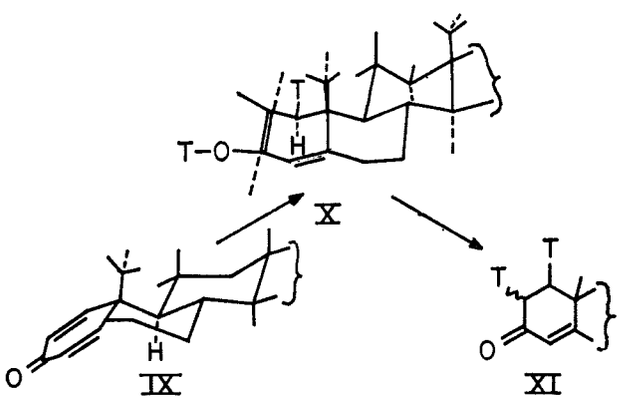
(20) S. Burstein, H. Kimball, and M. Gut, *Steroids*, **8**, 789 (1966).
(21) T. Morato, K. Raab, H. J. Brodie, M. Hayano, and R. I. Dorfman, *J. Amer. Chem. Soc.*, **84**, 3764 (1962).
(22) J. D. Townsley and H. J. Brodie, *Biochemistry*, **7**, 33 (1968).
(23) H. J. Brodie, G. Possanza and J. D. Townsley, *Biochim. Biophys. Acta*, **152**, 770 (1968).
(24) C. Djerassi and J. Gutzwiller, *J. Amer. Chem. Soc.*, **88**, 4537 (1966).
(25) H. J. Brodie, K. J. Kripalani and G. Possanza, *ibid.*, **91**, 1241 (1969).

droxyandrostan-17-one-5α,6α,7α-t[5] in which there was 0–3% exchange of tritium into stable[3,5,8] or enolic positions other than at a position α to the double bond being reduced.[5] After oxidation to androstenedione-1,2-t with negligible tritium loss, this material was incubated with a 10,000 × g supernate from *B. sphaericus*. The product, androsta-1,4-diene-3,17-dione, showed an apparent 57% loss of tritium (Table II). To be a true measure of tritium loss *due to conversion*, all tritium at C-2 would have to be eliminated, since 44% was at the unaffected 1β position. The 20% increase in the $^3H/^{14}C$ ratio of the recovered starting material suggests that the high *apparent* loss was due to the isotope effect operating at the C-1α and -2β positions,[26] retarding the conversion of tritiated (also at 1β and 2α) molecules to product. Further evidence for this conclusion was obtained when incubation of the androstenedione-1,2-t with a whole-cell preparation gave androsta-1,4-diene-3,17-dione, with only 38% loss of tritium. There was a much smaller 4% rise in the $^3H/^{14}C$ ratio in the recovered substrate, even though the percent conversion was appreciably higher. The reduced loss is apparently due to exchange which occurs in whole-cell preparations at the 2β and 1α positions,[26] decreasing the possibility of an isotope effect. The rates of exchange and dehydrogenation are competitive, so that some isotope effect of the type noted for the cell-free dehydrogenation is possible and the 38% loss represents a *maximum* amount of tritium at C-1α and C-2β. Since the tritium at the C-1α is 13% and the maximum amount at C-2 is 43%, the maximum amount at C-2β is 25% (38% total loss − 13% at C-1α); and by difference the amount at C-2α is 18%. This represents a tritium ratio 2β:2α of 1.4:1, much less than the 1β : 1α ratio of 3.4:1.

The 69% tritium loss on conversion of testosterone-1,2-t-4-^{14}C to estrone supports this conclusion. We have established that aromatization with placental preparations involves elimination of the 1β[21,22] and the 2β[25] hydrogens. Since the 1β position originally contained 44% of the tritium, the additional 25% loss may be assigned to the 2β position, confirming that the $\beta:\alpha$ tritium ratio at C-2 is 1.4:1 (25:18).[27–29] In the aromatization, the first step, C-19 hydroxylation, does not require the involvement of the hydrogens at C-1 and C-2; and the C-19 hydroxy intermediate then metabolizes rapidly and completely under our incubation conditions, minimizing any isotope effect that may operate in the latter stages of the transformation to estrogen. Thus, the tritium decrease obtained in this case should reflect that lost at C-1β and C-2β. In further support of this, we could not detect an isotope effect involving the C-1β proton loss[22] during aromatization of androstenedione; and in the present experiment the recovered testosterone substrate and its C-17 oxidized metabolite, androstenedione, had essentially the original ratio, suggesting little isotope effect or exchange at C-2 also. It should be noted, however, that when deuterium labeling was used some exchange was observed.[25]

The large difference in the $\beta:\alpha$ ratio of tritium at C-1 and C-2 for testosterone 1,2-t, and perhaps the lower amount of tritium at C-2 compared with C-1, especially in the reduction of prednisolone (I) (see, however, reference 6), shows that the mechanism of C-1,2 hydrogenation of the $\Delta^{1,4}$-3-one system does not involve complete *cis* addition. The results support a mechanism involving 1,4 addition[30] to the Δ^1-3-one system. Inspection of Dreiding models suggests that the initial reduction at C-1 occurs predominately β because the planar structure of ring A (IX) is angled away from the C-10 methyl group and toward the C-9 hydrogen. A 1,4 addition gives X, which would probably accept hydrogen (tritium) at C-2 either from the catalyst surface or from the C-3 hydroxyl on collapse of the enol. In the addition to X, α attack appears to be favored, since the axis of the C-2 double bond is bent toward the C-10 methyl group. However, the steric preference for α attack might be opposed by an electronic factor favoring β (axial) addition. For either mechanism it is expected that the $\beta:\alpha$ ratio of tritium at C-2 would be less than at C-1 to the extent that 1,4 addition occurs.

SCHEME II



Experimental Section

Materials.—Testosterone-1,2-t (prepared[31] as described[9]) and testosterone-4-^{14}C were obtained from New England Nuclear Corporation. They were converted to androstenedione with chromic–sulfuric acid in acetone[17] (0–5°, 10 min). Prednisolone (I) was obtained commercially, and 11β-hydroxyandrosta-1,4-diene-3,17-dione (II) was prepared from it by sodium bismuthate oxidation.[10] Substrates for double-label experiments were purified separately by chromatography and then were combined to give the desired count ratio. The ratios remained constant when samples of the materials were purified by chromatography and crystallized following reverse isotope dilution.

Procedures involving solvents, chromatography, scintillation counting, ir, and uv spectroscopy have been reported.[8,22] Systems for chromatography are recorded as per cent by volume.

Reduction with Tritium Gas.[32]—To the solution of 0.1 mol of prednisolone (I) or 11β-hydroxyandrosta-1,4-diene-3,20-dione (II) in 3 ml of dioxane was added 20 or 30 mg of 10% palladium–charcoal and 0.1 or 0.15 mmol of carrier-free tritium gas re-

(26) R. Jerussi and H. J. Ringold, *Biochemistry*, **4**, 2113 (1965).

(27) In aromatizing 1,2-tritiated substrates, Bolte, *et al.*,[28] found a 56% loss of tritium in estrone after a placental perfusion; and Axelrod and Goldziehe,[29] with preparations of minced normal and polycystic ovaries, observed tritium changes of ~0 to −67% in several estrogen metabolites. Assuming that the substrates had tritium distributions similar to those reported here, it appears that the preparations and conditions affect the % tritium change; of course, this does not necessarily mean that different stereochemical mechanisms for estrogen biosynthesis are operating. The value closest to ours for tritium loss during aromatization (67 *vs* 69%) occurred with a pregnancy ovary where, as in our incubations, there was apparently an appreciable conversion to product.

(28) E. Bolte, S. Mancuso, G. Ericksson, N. E. Wiqvist, and E. Diczfalusy, *Acta Endocrinol.* (Copenhagen), **45**, 535 (1964).

(29) L. R. Axelrod and J. W. Goldzieher, *J. Clin. Endocrinol. Metab.*, **25**, 1275 (1965).

(30) H. Simon and O. Berngruber, *Tetrahedron Lett*, 4711 (1968), and references therein.

(31) L. Geller, New England Nuclear Corp., personal communication, 1968.

(32) For the precise procedure see K. L. Laumas and M. Gut, *J. Org. Chem.*, **27**, 314 (1962).

spectively. After stirring for 4 hr, the mixture was purified as indicated below.

11β,17,21-Trihydroxypregn-4-ene-3,20-dione-1,2-*t* (III).—Chromatography of the crude product on a Celite partition column, using methanol–water (1:1) as stationary phase and benzene as mobile phase, gave 700 mCi of product corresponding to R_f = 0.22.

11β-Hydroxyandrost-4-ene-3,17-dione-1,2-*t* (IVa).—Similar chromatography, with the stationary phase methanol–water (4:1) and the mobile phase toluene–ligroin (2:1), yielded 950 mCi, corresponding to R_f = 0.6. Specific activity data on the following transformations of 11β-hydroxy compounds are given in Table III, next to the appropriate numbers.

Base Equilibration.—11β-Hydroxyandrost-4-ene-3,17-dione-1,2-*t* (IVa, IVb) was diluted with carrier and refluxed under nitrogen for 2 hr with 0.25 *N* KOH in 67% aqueous methanol. The equilibrated materials Va and Vb were purified by tlc in 20% ethyl acetate–benzene and crystallized from acetone to constant specific activity. The equilibrations and purifications were repeated until constant specific activity was obtained (Table III, no. 2, 3, 9, 10, and 11).

C-1,2 Dehydrogenation with *B. sphaericus* (ATCC 7055) (VIa and VIb from Va and Vb).—After a 400-ml culture of *B. sphaericus* was grown for 48 hr,[33] approximately 135 mg of Va or Vb. (no. 4, 12) was added. After further incubation for 12 hr, VI was isolated, purified by preparative tlc in 20% acetone–benzene, and crystallized from acetone to constant specific activity (no. 4–5 and no. 12–13). Physical constants: mp 185–186°, $[\alpha]^{25}D$ (acetone) 120°, λ_{max}^{EtOH} 243 mμ (ε 14,950) (lit.[34] mp 181–182°, $[\alpha]^{25}D$ 138°, λ_{max}^{EtOH} 242 mμ (ε 15,200); ir identical with that of reference standard.

C-1,2 Dehydrogenation with DDQ (VIc from Vb).—A mixture of 170 mg of Vb (no. 12), 91 mg of 2,3-dichloro-5,6-dicyanoquinone (Aldrich, recrystallized from benzene), and 18 ml of benzene was refluxed for 20 hr. An additional 45 mg of reagent was added and refluxing was continued for another 10 hr. After filtering and washing the benzene with bicarbonate and water, evaporation gave 159 mg of crystals. Preparative tlc in 15% benzene–ethyl acetate and recrystallization as above gave VIc (no. 14) with double mp[35] 108–115° and 186–187°, as well as the other physical constants noted in the preceding section.

Androsta-1,4-diene-3,11,17-trione (VII) from 11β-Hydroxyandrosta-1,4-diene-3,17-dione-1-*t* (VI).—An acetone solution of 102 mg of VIb and VIc (no. 13 and 14) was treated with 1.6 ml of the Jones reagent[17] for 15 min at room temperature and then poured onto ice. After extraction with ethyl acetate and washing with aqueous bicarbonate and water, evaporation gave 87 mg of crystals. No loss of tritium was realized in the formation of this compound or after equilibration with base as above (no. 15). VIa was converted to VIIa in a similar fashion.

3β-Acetoxy-1-methylestra-1,3,5(10)-triene-11,17-dione (VIIIa and b) from Androsta-1,4-diene-3,11,17-trione (VIIa and b).[18]—To 70 mg of VIIa or VIIb (no. 15) dissolved in 2 ml of acetic anhydride was added 0.21 ml of a solution of 0.125 ml of 70% perchloric acid in 10 ml of acetic anhydride. The solution was allowed to stand at room temperature for 5 hr. After column and thin layer chromatography on silica gel, crystallization to constant specific activity gave materials no. 7 and 16, mp 203–210° (lit.[18] mp 203–208°) and with ir and uv spectra in agreement with the values reported. Approximately 10 mg was refluxed with base as above for 2 hr. Aliquots were taken before and after treatment, neutralized with HCl, and evaporated to dryness. The radioactivity was determined by scintillation counting. Before base treatment, the activity was 447 cpm, and after treatment, 443 cpm.

Deuterium incorporation into 11β-Hydroxyandrost-4-ene-3,17-dione.—Clean metallic potassium was treated with CH_3O-*d* (98%) and then diluted with 99.9% deuterated water to give 20 ml of a 2% base solution (w/v) in 50% aqueous methanol. To this was added 25 mg of steroid, and the mixture was refluxed under nitrogen for 4 hr. The mixture then was cooled, neutralized, and extracted with ethyl acetate. The dried residue was dissolved in methanol and evaporated to dryness several times to remove deuterium from the 11-hydroxyl group. Following purification by tlc and crystallization, combustion analysis (J. Nemeth, Urbana, Ill.) showed that it contained 24.94 atom % excess deuterium corresponding to 6.5 atoms per molecule.

TABLE III

STEREOCHEMICAL ANALYSIS OF TRITIUM IN 11β-HYDROXY COMPOUNDS. RECRYSTALLIZATION DATA

No.	Compd	Recrystn no.	dpm, μmol	Average
	Experiments with Prednisolone (I)			
1	IVa	1	24,700	
		2	26,300	
		3	25,300	25,400
2	Va (base equil of IVa)	1	21,400	
		2	21,600	21,500
3	Va (2nd equil of IVa)	1	20,300	
		2	19,900	20,100
4	Va (substr for *B. sph.*)	1	30,200	
		2	28,200	
			30,200	29,500
5	VIa	1	23,600	
			23,800	23,700
6	VIa (substr for VIa to VIIIa)	1	28,600	...
7	VIIIa	1	4,320	
		2	4,550	
			4,230	4,370
	Experiments with 11-β-hydroxyandrosta-1,4-diene-3,17-dione (II)			
8	IVb	1	77,900[a]	
		2	68,500	
		3	72,300	70,400
9	Vb (1st base equil of IVb)	1	41,800	
		2	47,500	
		3	48,400	47,900
10	Vb (2nd equil)	1	40,000	
		2	43,700	41,900
11	Vb (3rd equil)	1	40,900	
		2	41,300	41,100
12	Vb (for conversion to VIb and VIc)	1	11,900	
		2	12,200	12,000
13	VIb (*B. sph.*)	1	9,200	
		2	8,500	
		3	9,200	9,230
14	VIc (DDQ)	1	9,430	
		2	9,180	
		3	9,310	9,300
15	VIIb (from no. 13 and 14 followed by base treatment)	1	9,350	
		2	9,620	9,480
16	VIIIb	1	1,520	
		2	1,420	1,460

[a] Underlined values *not* used to compile average.

Testosterone-1,2-*t*.—Analysis carried out as previously reported[3,8] showed that 44% of the tritium was at C-1, 13% was at C-1α, and 43% was enolizable.

Incubations. 1. *B. sphaericus*.—The whole-cell incubation was carried out as detailed above, using 50 mg of androstenedione-1,2-*t*-4^{14}C, sp activity 7 × 10^4 dpm/mg ^{3}H and 2.96 × 10^3 dpm/mg ^{14}C (ratio 23.6:1) per 400 ml of culture medium. After extraction and preparative tlc in 28% acetone–hexane, androsta-1,4-diene-3,17-dione product and unconverted androstenedione were eluted. Conversion to product was 98%, judged by the relative amount of ^{14}C associated with the two zones. The $\Delta^{1,4}$ product was crystallized to constant ^{3}H and ^{14}C activities; ^{3}H/^{14}C ratio, first crystallization, 14.7:1; second, 14.4:1. The recovered substrate was purified successively in the ligroin–propylene glycol and Bush A paper systems; ratios, 24.7:1, 24.4:1.

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The cell-free incubations were carried in five 25-ml conical flasks for 6 hr at 30° using 2.5 mg of substrate with similar specific activities (ratio 25.3:1), 1 mg of menadione, and 5 ml of enzyme solution per flask.[26] After extraction and preparative tlc as above, androsta-1,4-diene-3,17-dione product and unreacted starting material were eluted; conversion was 23%. They were diluted with appropriate carriers and crystallization to constant specific activities and ratios: product, 10.8:1, 10.7:1; substrate, 29.5:1, 30.8:1.

2. **A placental incubation**[22] was carried out with a 10,000 × g supernate preparation prepared in phosphate buffer using 200 μg of testosterone-1,2-t (sp activity 2.5 × 10^4 dpm ^3H and 944 dpm ^{14}C per μg, ratio 26.5) per 20 g wet weight of tissue. After incubation in air at 37° for 1 hr in the presence of an NADPH generating system, the mixture was extracted with ethyl acetate. The extract was chromatographed in the ligroin–propylene glycol paper system for 12 hr, and then in the toluene–propylene glycol systems without elution. The material in the estrone–testosterone and androstenedione areas were purified further by tlc. The $^3H/^{14}C$ ratios in dpm of the estrone were 8.2:1 after tlc in 20% ethyl acetate–benzene, 8.6:1 after a second crystallization, 8.1:1 after partition between KOH and toluene, and 8.3:1 after thin layer chromatography and two crystallizations of the acetate. The conversion to estrone was about 40%, as judged from the radioscans of the chromatograms.

The testosterone after tlc, reverse isotope dilution, and recrystallization showed $^3H/^{14}C$ ratios of 27.0:1, 27.7:1, and 28.0:1. The androstenedione material after tlc in 20% ethyl acetate–benzene followed by reverse isotope dilution and crystallization after $^3H/^{14}C$ ratios of 26.5:1, 25.8:1, and 26.5⁻1. Results in this and the preceding section are summarized in Table II.

Registry No.—I, 50-24-8; II, 898-84-0; tritium, 10028-17-8.

Acknowledgment.—We are grateful to Mrs. Cynthia E. Hay for skilled technical assistance.

Chemistry of 3-(N-Acetylureido)-4,5-oxidoandrostan-17β-ol Acetates[1a]

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Received January 20, 1969

Epoxidation of 3-ureido-Δ⁴-androsten-17β-ol derivatives **1a** and **6a** afforded exclusively the *cis*-4,5-oxido derivatives **2a** and **7a**, whereas the unsaturated N-acetylureido compounds yielded a mixture of oxides with predominant formation of the *cis* oxides. The *trans*-ureido oxides **3** and **8** were prepared from the *cis*-3-hydroxy 4,5-oxides **4** and **10**. The hydroxyl group was epimerized through the mesylates to the *trans*-azido oxides **5** and **11**. Reduction of the azides with hydrazine hydrate gave the amines which were converted into the ureides with nitrourea. Dilute acid-catalyzed treatment of the *cis*-3-(N-acetylureido) oxides **2b** and **7b** proceeded slowly to the *trans*-diaxial opened products **12** and **13**. Neighboring-group participation of the N-acetylureido group was realized in the acid treatment of the *trans* oxides **3** and **8**.

In continuation of the study on the chemistry of C-3 ureido steroids,[2,3] the synthesis of isomeric 3-ureido- and 3-(N-acetylureido)-4,5-oxidoandrostanes and the participation of the ureido group on ring opening of the epoxide have been investigated.

Epoxidation of 3α-ureido-Δ⁴-androsten-17β-ol acetate (**1a**) gave almost exclusively the *cis* product, 3α-ureido-4α,5-oxido-5α-androstan-17β-ol acetate (**2a**), whereas the 3α-N-acetylureido derivative **1b** gave the epimeric α- and β-epoxides **2b** and **3b** in a 2:1 ratio. The α-epoxide **2b** could also be prepared from 3α-ureido-Δ⁴-androsten-17β-ol (**1c**) and *m*-chloroperoxybenzoic acid followed by acetylation. The configuration of the epoxides was assigned on the basis of nmr spectrometric evidence.[4] The proton at C-4 in both **2a** and **2b** appeared as a doublet at δ 3.01 (J = 4.5 cps) indicating an epoxide ring *cis* to the substituent at C-3. In **3b** the C-4 proton appeared as a singlet at δ 2.85, demonstrating the *trans* relationship of the epoxide with the C-3α-N-acetylureido group. The stereoselective introduction of the epoxide *cis* to the C-3 ureido group can be ascribed to the hydrogen bonding between $-N^1H$ and the carbonyl group of the peracid directing the reagent from the *cis* face of the steroid nucleus. Henbest and Wilson[5] have proposed such a transition complex for the stereoselective epoxidation of cyclic allyl alcohols. It has also been found that cyclic allyl acetamido and benzamido groups have similar strong directive influence on epoxidation.[6,7] The formation of an appreciable amount of the *trans* epoxide from the 3α-N-acetylureido derivative **1b** may be in part due to steric hindrance of the *cis* face by the bulkier group as well as preferential hydrogen bonding of the reagent with the more acidic $-N^3H$ placing the peracid in a less favorable position for epoxidation; consequently a larger proportion of the *trans*-β epoxide **3b** could be formed.

In order to prepare larger amounts of the *trans*-β epoxide **3**, the method employed by Ponsold[8] for the preparation of 3α-acetamido-4β,5-oxido-5β-cholestane was employed. The 4β,5β epoxide **4a** was stereoselectively introduced by *m*-chloroperoxybenzoic acid oxidation of Δ⁴-androstene-3β,17β-diol 17-monoacetate derived from testosterone acetate. Epimerization at C-3 was accomplished by mesylation to **4b** and treatment with sodium azide to give 3α-azido-4β,5-oxido-5β-androstan-17β-ol acetate (**5**). The α orientation of the azido group was demonstrated by the singlet at δ 2.88 due to the proton at C-4, indicating a *trans* relationship of the epoxide and the azido group.[4] Reduction of the azide with hydrazine hydrate in the presence of Raney nickel afforded the amine, which was converted to 3α-ureido-4β,5-oxide-5β-androstan-17β-ol acetate (**3a**) with nitrourea.[2] Attempts to form the N-acetyl derivative (**3b**) were unsuccessful.

(1) (a) This investigation was supported by a grant from the American Cancer Society and Grant CA-07304 from the National Cancer Institute, National Institutes of Health, U. S. P. H. S. (b) Visiting Scientist, 1966–1967. Institut de Chimie des Substances, Naturelles, Gif-sur-Yvette, France.

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