The more likely pathway for C-1 hydrogen elimination would involve either displacing it by an activated group "Y" (for example hydroxyl) which can later be eliminated[9] (pathway II) or eliminating it as a hydride ion concerted with the removal of the C-19 oxygenated group (pathway III). This latter pathway could take place either through the mechanism shown[10] or by a four-center type (cyclic) elimination involving an activated C-19 oxonium ion intermediate (not shown.) From present knowledge, these types of reactions could reasonably be expected to involve TPNH and oxygen and the 1β-hydrogen. Experiments are now in progress to define the more likely pathway.[11]

Acknowledgment.—We wish to thank Drs. H. J. Ringold and M. Gut for their active interest in various aspects of this problem.

(9) The group "Y" also could be eliminated in concert with the C-10 group to form a C-1(10) double bond.

(10) This is similar to a mechanism previously proposed (M. Hayano, H. J. Ringold, V. Stefanovic, M. Gut and R. I. Dorfman, *Biochem. Biophys. Research Commun.*, **4**, 454 (1961)) except that it involves the 1β-hydrogen.

(11) This work was supported in part by U. S. Public Health Service grants A-2672, FF-258, and training grant CRTY-5001.

(12) Trainee in the Training Program for Steroid Biochemistry.

(13) Post-doctorate Trainee in the Training Program for Steroid Biochemistry.

TOMAS MORATO
KARL RAAB[12]
HARRY J. BRODIE[13]
MIKA HAYANO
RALPH I. DORFMAN

WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS.

RECEIVED AUGUST 1, 1962

THE STEREOCHEMICAL COURSE OF THE CATALYTIC HYDROGENATION OF RING A UNSATURATED STEROIDS

Sir:

In the reactions of double bonds in ring A of steroids, attack usually occurs predominantly on the alpha face since this is often less hindered due to the presence of the 10β-methyl group.[1] Indeed, some workers have made "alpha face attack" a general rule for, in a recent publication,[2] it was assumed, based on the type of work cited in reference 1, that in the reduction of $\Delta^{1,4}$ steroids with tritium on palladium catalyst, the label goes mostly into the 1α and 2α positions.

For the study of the mechanism of estrogen biosynthesis, we wished to prepare a C-1 tritiated androst-4-ene-3,17-dione of known configuration. To do this 17β-hydroxy-androsta-1,4-dien-3-one was reduced with tritium gas over palladium.[3] We can report now that under these conditions the attack at C-1 is predominantly *beta* in a ratio of about 3:1. In contrast, however, when androst-1-ene-3,17-dione is reduced in the same manner the introduction at C-1 is over 90% alpha as expected.

(1) For examples of alpha attack during reduction, epoxidation and glycol formation, see L. F. Fieser and Mary Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 254, 266, 271–274. The less common beta attack has been shown in hydrogenations of Δ^4 and Δ^7 compounds where a ring junction is involved (pp. 272, 274).

(2) L. R. Axelrod and J. W. Goodzieher, *J. Clin. Endocrinol. and Metabolism*, **22**, 537 (1962).

(3) Compare P. Osinski aud H. Vanderhaeghe, *Rec. trav. chim.*, **79**, 216 (1960).

The proof for the distribution of tritium in the two reduced products is outlined in the accompanying flow sheet. Before determining the distribution of tritium at C-1, the tritium at C-2 and other labile positions was removed by refluxing with KOH in aqueous methanol. Experiments have shown that the percentage of tritium lost from these positions ranges from 40 to 60%.[4] For clarity, tritium is shown at the positions of highest concentration in the reduced compounds III, IV, and V. All reductions were carried out with tritium gas over 5% palladium–charcoal in dioxane solvent. The structures of all compounds were proven from mixture melting points and by comparing their infrared spectra with authentic samples. The radiochemical purity was established by purifying to constant specific activity using paper chromatography and recrystallization.

Androst-1-ene-3,17-dione (I) was reduced and then refluxed with potassium hydroxide in aqueous methanol to give compound III. Compound III then was treated with dichlorodicyanoquinone (DDQ)[5] and incubated with *Bacillus sphaericus* (ATCC 7055)[6] to give compound IIIA and IIIB with losses of 89 and 93% of the tritium, respectively. This showed that the reduction and subsequent dehydrogenation were quite stereospecific for addition and removal of tritium at C_1. That the removal of tritium was from the alpha position was indicated from the literature on these reactions[5,6] and was proven conclusively in a recent study by Ringold, Gut, Hayano and Turner,[7] who showed that with 5α-androstane-3,17-dione-1α-H^2 the 1α-hydrogen (deuterium) is lost exclusively. They also showed that the reduction of the androst-1-ene-3,17-dione with deuterium proceeds almost exclusively alpha at C-1.

To determine whether the enzymatic and DDQ reactions were as specific for the elimination of the 1α-hydrogen with a C-4 double bond present III was oxidized to compound IV using the standard bromination–dehydrobromination technique.[8] Compound IV then was oxidized to compound IVA with DDQ. Here 76% of the tritium was lost, indicating that some of the 1β-hydrogen was eliminated with the C-4 double bond present. Dehydrogenation with *B. sphaericus* gave the same result as with the saturated compound, *i.e.*, loss of 93% of the tritium (IVB), showing that in this reaction the 1α-hydrogen is lost preferentially with the Δ^4-compound to the same extent as with 5α-androstane-3,17-dione.

Finally, 17β - hydroxy - androsta - 1,4 - dien - 3-one (II) was reduced catalytically to give a mixture of products.[3] The Δ^4-compound was isolated

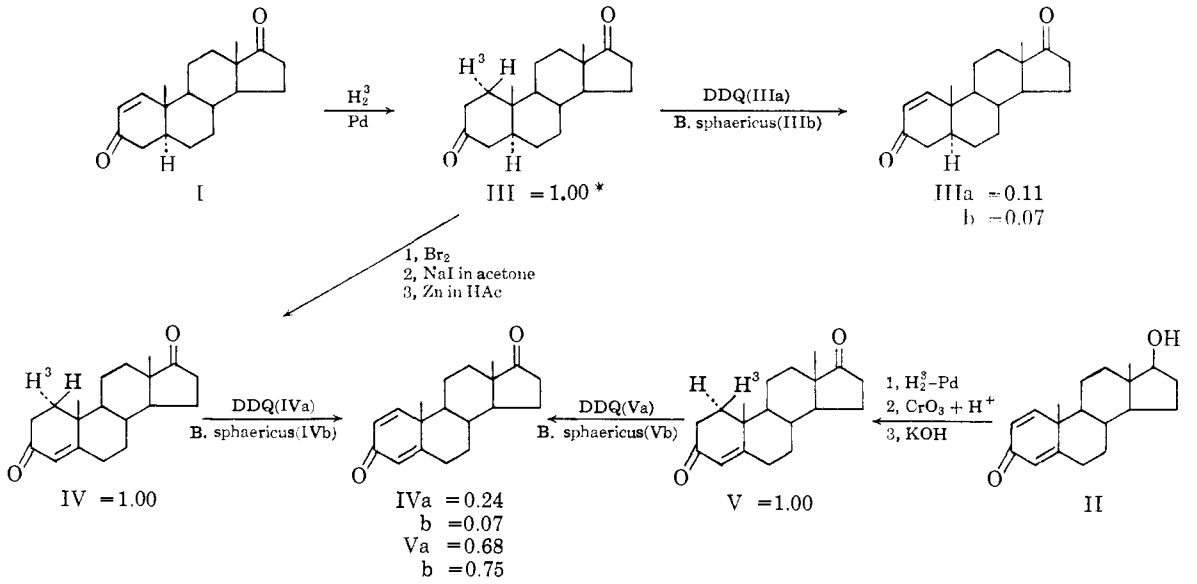
(4) M. Gut and M. Hayano in "Advances in Tracer Methodology," Vol. I, S. Rothchild, ed., Plenum Press, New York 11, N. Y., in press. Osinski and Vanderhaeghe have reported (ref. 3) that after the reduction of the Δ^1 bond of the bismethylene dioxide of prednisone no loss of tritium was noted on treatment with potassium hydroxide. As can be seen, this is contrary to results obtained with C-1,2-tritiated testosterone and androstenedione.

(5) H. J. Ringold and A. Turner, *Chem. and Ind.*, 211 (1962).

(6) M. Hayano, H. J. Ringold, V. Stefanovic, M. Gut and R. I. Dorfman, *Biochem. Biophys. Res. Comm.*, **4**, 454 (1961).

(7) H. J. Ringold, M. Gut, M. Hayano and A. Turner, *Tetrahedron Letters*, in press.

(8) G. Rosenkranz, O. Mancera, V. Gatica and C. Djerassi, *J. Am. Chem. Soc.*, **72**, 4077 (1950).



* Numbers refer to the relative specific activities of reactants and products.

from the mixture by paper chromatography, oxidized to the diketone using the Jones method[9] and equilibrated with base to give V. Compound V was oxidized both with DDQ to give compound VA (68% retention of tritium) and with *B. sphaericus* to give compound VB (75% retention of tritium). The remaining tritium was shown to be at C-1 by oxidizing another sample of compound V to androsta-1,4,6-triene-3,17-dione using chloranil[10] and then DDQ.[5] When this compound was treated with acid it rearranged to 1-methylestrone acetate which was inactive (specific activity<0.01%). In this reaction, the methyl group originally at C-10 displaces the remaining hydrogen at C-1 (dienone-phenol rearrangement[11]).

Because the oxidation with *B. sphaericus* is essentially specific for the elimination of the 1α-hydrogen, this reaction shows that the previous reduction of androst-1-ene-3,17-dione with tritium to the saturated compound gives 93% 1α-tritium and 7% beta and the reduction of the $\Delta^{1,4}$ compound gives 25% 1α-tritium and 75% beta. That androsta-1,4-diene-3,17-dione is reduced preferentially from the beta side probably is due to the planarity of ring A resulting from the dieneone structure. A consideration of Dreiding models shows that the planar ring A is tilted downward away from the beta angular methyl group at C-10 and toward the alpha axial hydrogen at C-9. Thus approach of catalyst may be hindered more by the C-9 hydrogen on the alpha face than by the methyl group on the beta face.

Reductions of $\Delta^{1,4}$ compounds further substituted on the beta face, *e.g.*, Δ^1-cortisol in which there is a hydroxyl group at C-11 indicates that here the alpha face may be attacked preferentially.[12] Thus for the reduction of $\Delta^{1,4}$ compounds it is difficult, *a priori*, to predict on which side of the molecule reduction will occur to the greater extent. Accessibility to either face can be changed by slightly modifying the molecule. A study of the effect of various groupings on the steric course of the reduction is being carried out.

Acknowledgment.—This work was supported in part by U. S. Public Health Service grants A-2672, A-3419 and Training Grant CRTY-5001.

(9) A. Bowers, T. G. Halsall, E. R. H. Jones and A. J. Lemin, *J. Chem. Soc.*, 2554 (1953).

(10) E. J. Agnello and G. D. Laubach, *J. Am. Chem. Soc.*, **82**, 4293 (1960).

(11) Ref. 1, p. 328.

(12) Inspection of a Dreiding model shows severe non-bonded interaction between the 11β-hydroxyl and the 10β-methyl group which may force the latter to bend closer over ring A, thereby preventing reduction of the C-1,2 double bond from the beta face.

(13) Post-doctorate trainee in The Training Program for Steroid Biochemistry.

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
SHREWSBURY, MASS.

HARRY J. BRODIE[13]
MIKA HAYANO
MARCEL GUT

RECEIVED AUGUST 1, 1962

SIGNS OF PROTON COUPLING CONSTANTS

Sir:

The recent findings[1] of opposite relative signs of J_{vic-HH} and J_{gem-HH} in typical ethane derivatives are in disagreement with the theoretical predictions[2] that both constants should be positive. Since the experimental and calculated coupling constants are in numerical agreement, it is not immediately obvious where the error lies.

The calculations, particularly of J_{gem}, involve small differences between large terms, and therefore may not be reliable. On the other hand, calculations[3] of magnitudes and signs of the larger coupling constants of directly bonded nuclei (*e.g.*, C^{13}-H) should be more secure. It was therefore of interest

(1) R. R. Fraser, R. U. Lemieux and J. D. Stevens, *J. Am. Chem. Soc.*, **83**, 3901 (1961); F. Kaplan and J. D. Roberts, *ibid.*, **83**, 4666 (1961); F. A. L. Anet, *ibid.*, **84**, 1053 (1962); A. McLauchlan and D. H. Whiffen, *Proc. Chem. Soc.*, 144 (1962); C. A. Reilly and J. D. Swalen, *J. Chem. Phys.*, **35**, 1522 (1961).

(2) M. Karplus and D. H. Anderson, *ibid.*, **30**, 6 (1959); H. S. Gutowsky, M. Karplus and D. M. Grant, *ibid.*, **31**, 1278 (1959); M. Karplus, *ibid.*, **30**, 11 (1959).

(3) M. Karplus and D. M. Grant, *Proc. Nat. Acad. Sci.*, **45**, 1269 (1959); N. Muller and D. E. Pritchard, *J. Chem. Phys.*, **31**, 768, 1471 (1959); J. N. Shoolery, *ibid.*, **31**, 1427 (1959); N. Muller, *ibid.*, **36**, 359 (1962).