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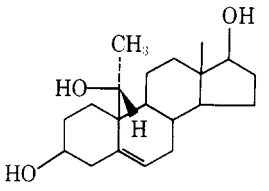
Reassignment of the Absolute Configuration of 19-Substituted 19-Hydroxysteroids and Stereomechanism of Estrogen Biosynthesis

Sir:

We wish to report evidence for reversing the previous assignment of the absolute configuration at the C-19 position of 19-hydroxy-19-methylandrost-5-ene[1] and of other structures[2] fundamentally dependent on it for their stereochemical assignments.[3] Using numerous reaction sequences and stereochemical considerations of the hypothesis of Karabatsos,[4] a modification of Cram's rule,[5] of Cherest et al,[6] and of relative rates of 3,19-oxide formation over A-ring Wicha and Caspi[1] assigned the 19*R* configuration for the only isolated (90% yield) 3β,17β,19-trihydroxy-19-methylandrost-5-ene (**I**) from methyllithium reaction with 3β,17β-diacetoxy-5-androsten-19-al (**2a**) and 19*S* (**3**) for the only isolated alcohol obtained by lithium aluminum hydride reduction of 3β,17β-diacetoxy-19-methylandrost-5-en-19-one (**2b**). Based on Wicha and Caspi's studies, Skinner and Akhtar[2a] tentatively assigned the 19*R*-^{3}H configuration to the major product of ^{3}H-labeled sodium borohydride reduction of the aldehyde **2c**. They proposed that in estrogen biosynthesis the 19-hydroxylation of the 19-hydroxyandrogen occurs at the 19pro*S* hydrogen.[2a] Contrary to this mechanism we proposed[7] stereospecific 19pro*R* hydrogen removal in enzymic aromatization of 19-hydroxytestosterone, which is a more direct precursor for estrogen biosynthesis than a Δ^5-3β-ol,[8] based on the assignment of 19*S*-^{3}H configuration to the major product (90% pro*S*/10% pro*R*) of the labeling of Δ^4-3-oxo-19-al.

To reconcile the apparent conflict for the stereomechanism of enzymic aromatization the following are considered: (1) stereospecificity of the 19-hydroxylation by human placental androgen aromatase could be opposite for the substrates 19-hydroxy-Δ^5-3β-ol or 19-hydroxy-Δ^4-3-one; (2) stereoselectivity of the nucleophilic attack of methyllithium and sodium borohydride on the steroidal 19-aldehyde could be different enough to make the tentative assignment by Skinner and Akhtar invalid; or (3) either one of the two assignments must be reversed.

We have carried out further conformational studies using X-ray crystallography, isotope labeling, NMR, and enzymic reactions. We report here that the stereoselectivity of



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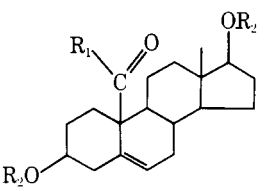
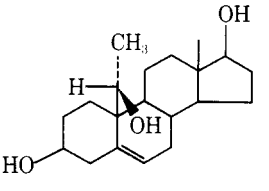


2a, $R_1 = H$; $R_2 = Ac$
b, $R_1 = CH_3$; $R_2 = Ac$
c, $R_1 = R_2 = H$



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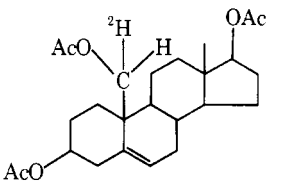

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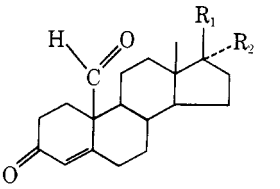
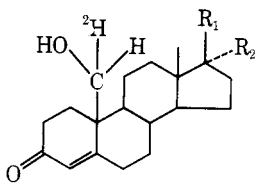
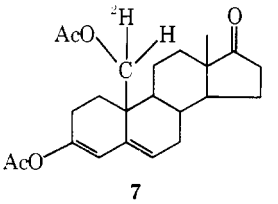


5a, $R_1 = OCOC_6H_5$; $R_2 = H$
b, $R_1, R_2 = O$



6a, $R_1 = OCOC_6H_5$; $R_2 = H$
b, $R_1, R_2 = O$



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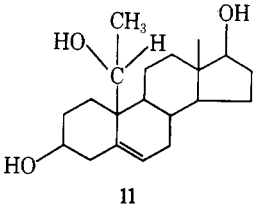

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the two nucleophilic reactions at the 19-aldehyde is similar, estrogen biosynthesis is by stereospecific removal of the 19pro*R* hydrogen of both the 19-hydroxylated Δ^5-3β-ol and Δ^4-3-one systems, and the previous configurational assignment[1,2] of 19-substituted 19-hydroxy-steroids should be reversed.

The aldehyde **2a** was reduced with deuterium labeled (>98% ^{2}H) sodium borohydride in methanol and the resulting alcohol was acetylated to give [19-^{2}H]-3β,17β,19-triacetoxyandrost-5-ene (**4**): mp 88–90°; ir 1735, 1240, 1030 cm^{-1}; ^{1}H NMR (60 MHz), δ 0.83 (18-CH$_3$, 3 H, s), 2.03 (OAc, 9 H, s). Quantitative analysis by ^{1}H NMR at 300 MHz,[9] as shown in Figure 1, gave 67% ^{2}H labeling at the higher field 19-proton (δ 3.96 ppm) and 33% at the lower field 19-proton (δ 4.49). 17β-Benzoyloxy-3-oxo-4-androsten-19-al (**5a**)[10] was similarly reduced with sodium borodeuteride to give [19-^{2}H]-19-hydroxytestosterone 17-benzoate (**6a**),[7,11] mp 242–245°. Analysis of the 19-protons by 300-MHz ^{1}H NMR showed 90% ^{2}H labeling at the lower field (δ 4.06) and 10% at the higher field proton (δ 3.89) in **6a** as in Figure 2. In order to establish the stereochemical relationships of the labels in the two systems, transformation of [19-^{2}H]-Δ^4-3-one to [19-^{2}H]-Δ^5-3β-ol was carried out without removal of the label. 3,17-Dioxo-4-androsten-19-al (**5b**)[12] was selectively reduced with sodium borodeuteride to afford [19-^{2}H]-19-hydroxy-4-androstene-3,17-dione (**6b**): mp 168–170°; ir, 3300, 1737, 1658, 1618 cm^{-1}; ^{1}H NMR ($CDCl_3$–D_2O), δ 0.92 (18-CH$_3$, 3 H, s), 5.91 (4-H, 1 H, s); 90% ^{2}H labeling at the lower field 19-H and 10% ^{2}H at the higher field 19-H as observed in **6a**. The unsaturated ketone was treated with acetic anhydride and perchloric acid to give [19-^{2}H]-3,19-diacetoxyandrosta-3,5-dien-17-one (**7**) as an oil. The enol acetate was

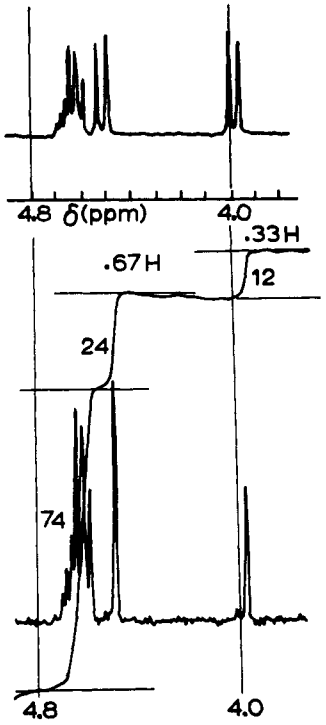


Figure 1. 3α,17α,19-Proton signals of 300-MHz ^{1}H NMR ($CDCl_3$) of 3β,17β,19-triacetoxyandrost-5-ene (top); and [19-^{2}H]-3β,17β,19-triacetoxyandrost-5-ene (**4**) (bottom), 19-pro*S* at δ 3.96 (0.33 H) and 19-pro*R* at δ 4.49 (0.67 H).

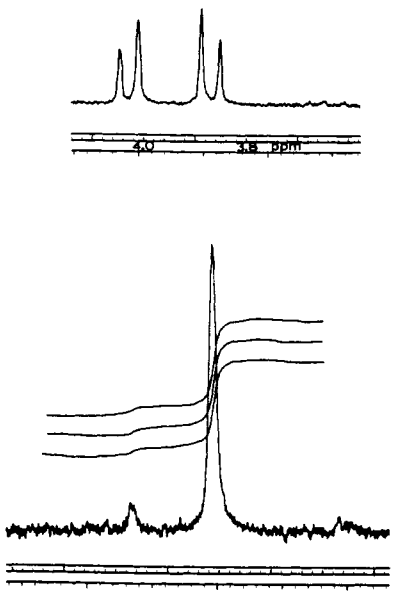


Figure 2. 19-Proton signals of 300-MHz ^{1}H NMR ($CDCl_3$-D_2O) of 19-hydroxytestosterone 17-benzoate (top) and [19-^{2}H]-19-hydroxytestosterone 17-benzoate (**6a**) (bottom), 19-pro*S* at δ 4.06 (0.10 H) and 19-pro*R* at δ 3.89 (0.90 H).

reduced with sodium borohydride in aqueous ethanol[13] and acetylated to give **4**, mp 88–90°. The 60-MHz ^{1}H NMR showed that the 90% ^{2}H-labeled 19-proton is now at δ 3.96, and 10% ^{2}H labeled at δ 4.49, identical positions with the 67% ^{2}H-labeled and 33% ^{2}H-labeled signals of the previous product, respectively. Thus the relatively upfield 19-hydrogen of **6a** corresponds to the relatively downfield 19-hydrogen of **4**. This establishes the same stereochemical predominance of asymmetric reduction of the 19-aldehyde of Δ^4-3-one and Δ^5-3β-ol systems. The stereoselectivity of the ^{2}H-labeling (67/33, 90/10 for Δ^5 and Δ^4, respectively) is also reflected in the ^{3}H labeling using the same reagent. The

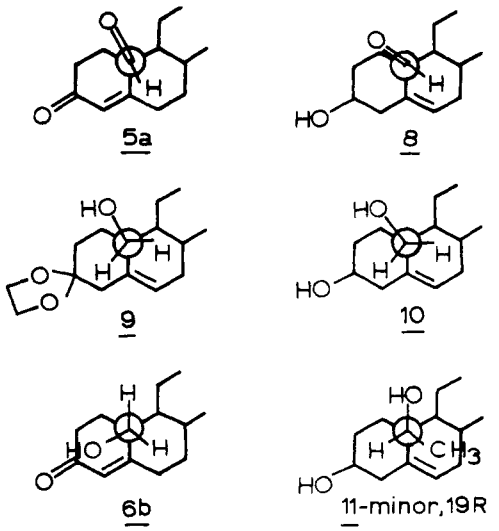


Figure 3. Conformation of the 19-group determined by X-ray crystallography of 17β-benzoyloxy-3-oxo-4-androsten-19-al (**5a**), 3β-hydroxy-17-oxo-5-androsten-19-al (**8**), 3-ethylenedioxy-5-androstene-17β,19-diol 17-benzoate (**9**), 5-androstene-3β,17β,19-triol 17-*p*-bromobenzoate (**10**), 19-hydroxyandrostenedione (6b), and [19*R*]-19-methyl-5-androstene-3β,17β,19-triol (**11**-*minor*).

ratio of labeled formic acid to water as formed by estrogen biosynthesis has been reported as 2 to 1[2a] and 9 to 1[7] from Δ^5 and Δ^4 precursors, respectively. Thus we can conclude that the mechanism of estrogen biosynthesis has the same stereospecificity of the 19-hydroxylation regardless of the precursor structures and isotope labeling.

Based on the conformational analyses of **5a** and **6b** (H instead of ^{2}H) by X-ray crystallography,[14] we have previously assigned[7] the 19pro*S* configuration to the major product of [19-^{2}H and ^{3}H] **6a**. In the crystal structure, the 19-aldehyde of **5a** is observed in the out-of-ring position equidistant from the 2β- and 11β-axial hydrogens and the C_1–C_{10}–C_{19}–O_{19} torsional angle is −30° (Figure 3). The 6β-, 8β-, 11β-, and 18-hydrogens obstruct approach to the aldehyde from the C-ring side. In addition the bowing of the flattened A ring toward the α-face exposes the aldehyde to easy approach by the reagent from over the A ring with only marginal obstruction provided by the 2β-axial hydrogen. However, the steric situation in the Δ^5-3β-ol series is different and it is difficult to draw a definite conclusion from the conformational observation. Crystal and molecular structures of 3β-hydroxy-17-oxo-5-androsten-19-al (**8**),[14] 3-ethylenedioxy-5-androstene-17β,19-diol 17-benzoate (**9**),[15] 5-androstene-3β,17β,19-triol 17-*p*-bromobenzoate (**10**),[15] and 19-hydroxyandrostenedione (**6b**)[14] have been solved using direct methods of X-ray crystallography and conformations around the C_{19}–C_{10} bond are shown in Figure 3. The 19-aldehyde of **5a** and **8** are observed in similar conformations as indicated by the C_1–C_{10}–C_{19}–O_{19} torsional angles of −30 and −4°, respectively.

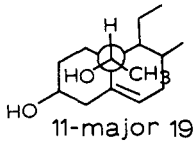
The 19 alcohol conformations observed in two Δ^5 structures **9** and **10** are nearly identical. Their out-of-ring conformations are defined by the C_1–C_{10}–C_{19}–O_{19} torsional angles of −38 and −34°, respectively. In contrast, the 19-alcohol in the Δ^4 structure **6b** is observed in the over-A-ring conformation. This conformation is favored because of the flexibility of the Δ^4-3-one system.[16] The Δ^4-3-keto-A-ring can flatten and bend to relieve β-face crowding.

The direction of attack on the aldehyde **2a** is difficult to predict since the 2β- and 4β-axial hydrogens on the A ring and the 8β- and 11β-axial hydrogens on the C ring give a similar steric situation on both sides of the aldehyde, in contrast to the one-sided situation in **5a**. The conformational analyses by X-ray crystallography vitiate the attempt of

Wicha and Caspi[1b] to predict the formation of the 19*R* configuration by stereochemical considerations. Nevertheless, one could predict a diminished stereoselectivity relative to that for Δ^4-3-one system. We have observed that the stereoselectivity of sodium borohydride reduction decreased from 9/1 to 2/1. Then, Wicha and Caspi's report[1] that the methyllithium reaction on **2a** gave a 90% yield of a single compound seems to be unpredictably stereoselective. In addition, the 19*R* assignment for the major methylated product now clearly contradicts our 19*S* assignment based on the conformational studies described above. For these reasons we undertook a methyllithium reaction of **2a** according to Wicha and Caspi.[1b]

Methyllithium reaction with **2a** gave over 90% yield of 19-methyl-5-androstene-3β,17β,19-triol (**11**) which showed one spot in TLC (ethyl acetate–ethanol, 9:1) exactly as described in the previous reports. This product, however, was separated into two distinct compounds in another TLC system (developed twice in chloroform–acetone, 8:2; ***11-major***, R_f = 0.50, and ***11-minor***, R_f = 0.44) and isolated in crystalline form in 63 and 24% yield, respectively. The ***11-major*** was recrystallized from acetone (mp 189–192°; ir, 1066, 1050, 1026 cm^{-1}; ^{1}H NMR (CD_3OD), δ 0.80 (18-CH_3, 3 H, s), 1.30 (19a-CH_3, 3 H, d, J = 7.0 Hz), 5.81 (5-H, 1 H, m); MS, *m/e* 302(M − 18), 276(M − 44), 258(276 − 18), 240(258 − 18), and 225(240 − 15)) and the ***11-minor*** recrystallized from aqueous methanol (mp 188–190°; ir, 1077, 1050, 1029 cm^{-1}; ^{1}H NMR (CD_3OD), δ 0.80(18-CH_3, 3 H, s), 1.29 (19a-CH_3, 3 H, d, J = 7.0 Hz) 5.81 (5-H, 1 H, m); MS, *m/e* 302(M − 18), 276(M − 44), 258, 240, and 225. Each showed no change in characteristics even after heating over its melting point. The ***11-minor*** was grown in single crystals from acetone and the crystal and molecular structure was solved.[17] The absolute configuration at the C-19 of ***11-minor*** was determined to be *R* and the conformation around the C_{10}–C_{19} bond showed that the 19-methyl function is at the over-B-ring and the oxygen at the out-of-ring position as shown in Figure 3. Thus the diastereomeric ***11-major*** to which 19*R* was previously assigned should be reassigned as 19*S*. The stereoselectivity of the nucleophilic attacks at the steroidal 19-aldehyde by the two reagents was thus found to be the same. The 19pro*R* hydrogen removal[7] in estrogen biosynthesis was confirmed.

Addendum. The absolute configuration of **11**-major was determined to be 19*S* by X-ray crystallography, after the submission of the manuscript. The conformation around the C_{10}–C_{19} bond showed that the methyl function is again at the over-B-ring and the oxygen at the over-A-ring position as shown below. The fact that both diastereomers have the methyl group at the over-B-ring position suggests that this is the least energy conformation. This offers an explanation for the cause of the reversal of the original assignment which relied on Wicha and Caspi's rationalization that the bulkiest of the three substituents on C-19, the methyl group, must be at the out-of-ring position for 3β,19-oxide formation.



11-major 19S

Acknowledgment. This investigation was supported by Research Grants HD04945 and CA10906 from the U.S. Public Health Service, and aided by the American Cancer Society Faculty Award PRA-72 (Y.O.). 3-Ethylenedioxy-5-androstene-17β,19-diol 17-benzoate was prepared by Dr. S. S. H. Gilani and 5-androstene-3β,17β,19-triol 17-*p*-bromobenzoate was synthesized by Dr. K. Yasuda for the crystallography study. Skilled technical assistance was provided by Mrs. Carol Yarborough and Mrs. Mary Erman. All this support is gratefully acknowledged.

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Comment on the Carbon-13 Nuclear Relaxation Measurements in Adenosine Monophosphate

Sir:

Recently Hamill, Pugmire, and Grant[1] reported in this journal the concentration dependence of carbon-13 nuclear relaxation data of 5′-AMP. The observed strong decrease of T_1 with concentration was explained by an increase of the stacking process with concentration. If this explanation is correct, one would expect a significant deviation from the simple Stokes-Einstein relation, namely, a deviation from slope 1 in a double log plot of T_1 vs. η^{-1}. In Figure 2 this plot is presented. The viscosity data for different concentrations were obtained by us (Figure 1) and combined with the ^{13}C relaxation data of Hamill et al.[1] Within the accuracy of the experimental results, the ^{13}C longitudinal relaxation times of the base carbons C-2 and C-8 can be explained by the simple Stokes-Einstein relation without invoking a change of particle size with concentration. This relationship, however, leaves undecided whether the stacking is already complete at the lowest concentration investigated. The following consideration excludes this possibility.

In molecules of the size of a nucleotide the relaxation of a carbon-13 bound directly to a hydrogen atom is completely described by dipole-dipole interaction between the carbon-13 and the proton spin.[2] Assuming isotropic motion of the