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Stereospecificity of Oxidation at C-19 in Oestrogen Biosynthesis

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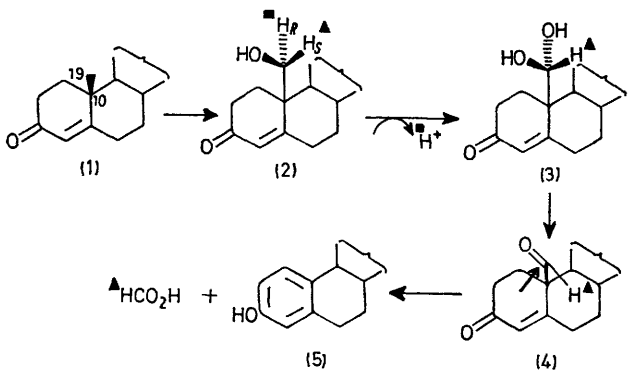
Summary A method has been devised for determining the configuration of samples of 19-acetoxy-Δ^5-androgens stereospecifically labelled at C-19; with the help of such samples it is shown that in the biological conversion of 19-hydroxyandrogens into oestrogen the *pro-R* hydrogen atom from C-19 is removed as a proton and the *pro-S* hydrogen atom is incorporated into formic acid.

It has been shown that in the biological conversion of the androgen (**1**) into oestrogen (**5**) the cleavage of the C-10–C-19 bond occurs at the oxidation level of an aldehyde with the removal of formic acid, as shown in the Scheme.[1,2] Enzymic studies have revealed that in the biosynthetic pathway the transformation of the alcohol (**2**) into the aldehyde (**4**) requires the participation of NADPH and O_2 thus implicating the involvement of the intermediate (**3**) or its equivalent in the oxidation.[2] This suggested that in the conversion of the alcohol (**2**) into oestrogen one of the C-19 hydrogen atoms may be liberated as water and the other released into formic acid. This facet has previously been examined by the following experiments.[2]

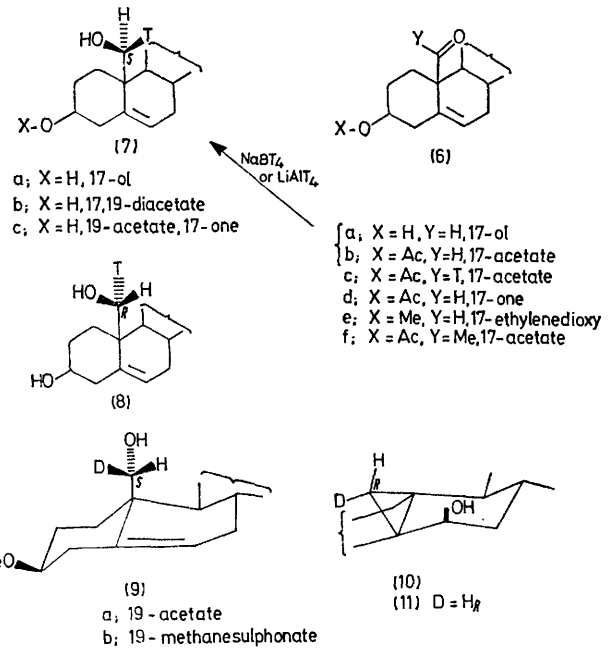
It was shown that the reaction of $NaBH_4$ with the 19-aldehyde group of compounds of the type (**6**, Y = ^{3}H or ^{1}H) occurs with a high degree of stereospecificity, thus permitting the synthesis of two tritiated alcohols (**7a**) and (**8**). The biological conversion of these alcohols into oestrogen by the sequence (**7a** or **8**) → (**2**) → (**3**) → (**4**) → (**5**) and determination of radioactivity in formic acid and water had revealed that in the oxidation step (**2**) → (**4**) one of the two hydrogen atoms at C-19 is stereospecifically removed.[2] Although the precise assignment of configuration to the new



Scheme. See footnote ‡ for explanation of the heavy arrow in (**4**).

chiral centre of (**7a**) and (**8**) was not made, attention was drawn to the stereochemical course favoured by Wicha and Caspi[3] for the reaction of MeLi with the 19-aldehyde group. Subsequent studies at Zürich have resulted in the development of a method[4] for the assignment of stereochemistry to

the two C-19 hydrogen atoms of compounds of the type (**9**). This information has now been used to extend our knowledge of the stereochemistry of the removal of C-19 hydrogen atoms in oestrogen biosynthesis.



R and *S* in structures (**7**)—(**11**) refer to the chirality at C-19.

The n.m.r. spectrum of a number of 19-acetoxy compounds of the type (**7b** and **7c**) has signals at δ *ca.* 4·53 (d, *J* 12 Hz) and *ca.* 3·97 (d, *J* 12 Hz) attributed to the two C-19 hydrogen atoms. When the androstenal (**6e**) was reduced with $LiAl^2H_4$ and the product converted by acid hydrolysis into the 19-deuterio-compound (**9**), the n.m.r. spectrum of the corresponding acetate (**9a**) had signals at δ 3·95 and 4·49 (0·2 and 0·8 H, respectively). Thus the reduction had occurred 80% stereospecifically. The methoxy-alcohol (**9**) was converted into the mesyl derivative (**9b**) which on solvolysis[5] furnished the deuterio-cyclopropane (**10**). It is known that the chemical shifts for the two cyclopropyl hydrogen atoms, H_R and H_S of (**11**), are at δ 0·35 and 0·89 respectively.[4,5] The deuteriocyclopropane (**10**) had in the n.m.r. spectrum signals at δ 0·89 (0·8H and 0·35 (0·2H), thus showing that in the compound (**10**) deuterium was predominantly in the H_R-position. On the well supported assumption that the cyclopropane ring in the compound (**10**) is formed by an S_N2 displacement mechanism then the precursor deuterio-methoxy-alcohol and its derivatives (**9**) must contain the deuterium in the *pro-S*-position. This implies that the doublets centred at δ 3·95 and 4·44 in the methoxy-acetate (**9a**) are due to the two C-19 hydrogen atoms in the *pro-S* and *-R* positions respectively. The position of these doublets is not affected by the nature of the C-3 substituents; accordingly, similar assignments can be made by analogy for other 19-acetoxy-compounds of type (**7b** and **7c**).

Since for biological work, 19-tritiated alcohols (**7a**) and (**8**) containing a free 3-hydroxy-group were needed, the stereochemical course for the reduction of the 19-carbonyl group of the 3β-acetoxy-19-aldehyde (**6d**) was studied using the n.m.r. correlation data described above. The latter aldehyde (**6d**) was reduced with NaB^2H_4 and the resulting product acetylated and partially hydrolysed to give in 30% overall yield compound (**7b**; D replaces T) which by mass spectrometric analysis was shown to contain two deuterium atoms. The n.m.r. spectrum of (**7b**) in the C-19 region had signals at δ 3·95 (0·28H) and 4·49 (0·72H) showing that the deuterium was predominantly in the *pro-S*-position. With this information available the Δ^5-aldehyde (**6a**) and its 3β,17β-diacetoxy-derivative (**6b**) were treated with NaB^3H_4 under the conditions used above to give two samples of the 19-tritiated alcohol (**7a**). The conversion of both of these samples into oestrogen by the sequence (**7a**) → (**2**) → (**3**) → (**4**) → (**5**) gave 62% of radioactivity associated with formic acid and 38% with water. Two additional samples of the 19-tritiated alcohol (**7a**) were prepared by the reduction of the Δ^5-aldehyde (**6a**) and its diacetate (**6b**) with $LiAl^3H_3$. Both these samples when converted into oestrogen released 70% of the radioactivity into formic acid and the remaining 30% into water. In contrast, it had been shown[2] previously that when the Δ^5-tritiated aldehyde (**6c**) was reduced with $NaBH_4$ and the alcohol (**8**) subjected to the biological conversion under the same conditions as those used for the alcohol (**7a**), the order of radioactivity was reversed; formic acid (24%) water (76%). Cumulatively, these results allow the following conclusions to be drawn. Firstly, the steric course for the reduction of the aldehyde group of (**6**) by $NaBH_4$ and $LiAlH_4$ is identical,† though the latter reagent offers a somewhat superior steric control. Secondly, it is the *pro-R*-hydrogen atom of (**2**) which is removed as H_2O during the oxidation step (**2**) → (**4**). This stereochemical conclusion is opposite to that tentatively indicated[2] on the basis of analogy with the MeLi reaction.[3],‡

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† Furthermore the stereochemistry of reduction is unaffected whether the C-3 carries hydroxy-, methoxy-, or acetoxy-groups.

‡ The procedure used by us, in this work and elsewhere,[2] for the reduction of the Δ^5-aldehydes (**6a** and **8**) has recently been extended to the reduction of the corresponding aldehydes in the Δ^4-3-one series (**4**). It was found that the alcohol (**2**,H_s = T) prepared by the reaction of (**4**) with NaB^3H_4, on biological conversion into oestrogen gave more radioactivity in formic acid than in water, as is the case with the Δ^5-alcohol (**7a**) prepared analogously. Thus stereochemically $NaBH_4$ must react with the aldehyde groups of the compounds (**4**) and (**6**) in a similar fashion. In Ref. 6 it was correctly hypothesised that in the chemical reduction of (**4**) into (**2**) the $NaBH_4$ derived hydrogen atom occupies the *pro-S*-position by invoking that the aldehyde group in (**4**) is fixed as shown in the structure (**4**) and the attack by the reducing agent occurs from the least hindered side (shown by the heavy arrow in structure **4**). However, the same principle when extended to either the reaction of MeLi with the aldehyde (**6**) or $LiAlH_4$ with the 19-methylketone (**6f**) will predict the opposite stereochemistry to that claimed in the literature.[3]

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[2] S. J. M. Skinner and M. Akhtar, *Biochem. J.*, 1969, **114**, 75.
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