Studies on the Removal of C-19 in Oestrogen Biosynthesis Using $^{18}O_2$

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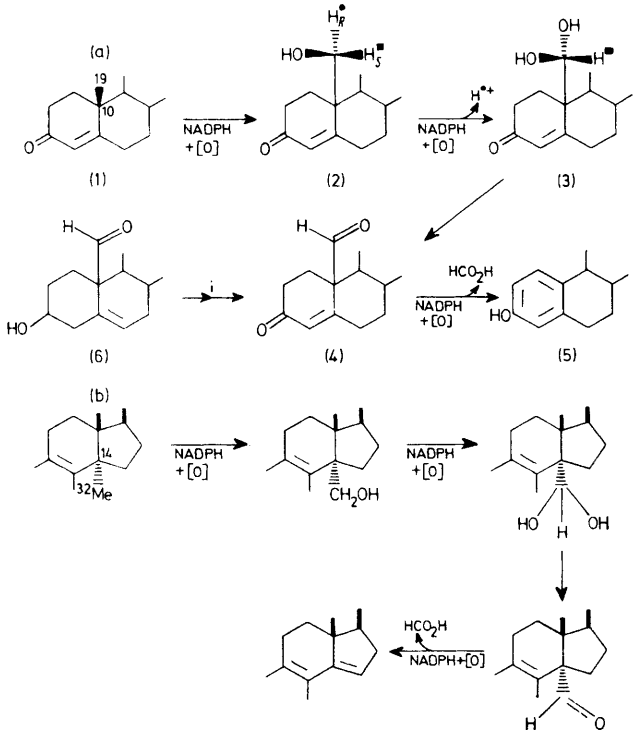
Summary Incorporation of ^{18}O into formic acid formed in the C-19 elimination, under $^{18}O_2$ gas, in oestrogen biosynthesis suggests the participation of a novel mechanism.

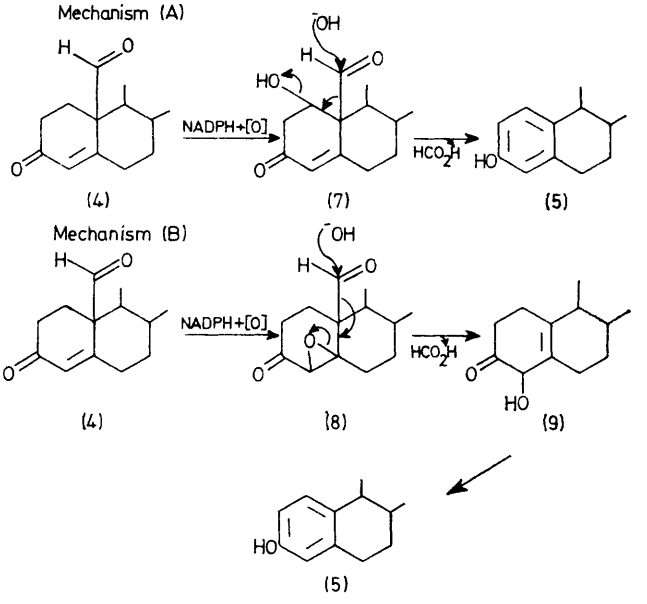
CURRENT information[1,2] on the pathway for the conversion of androgens into oestrogen is outlined in Scheme 1(a). We have shown that during the biosynthesis, the conversion of the 19-hydroxy compound (**2**) into the 19-aldehyde (**4**) requires the participation of NADPH and molecular oxygen, and that in this reaction one of the C-19 hydrogen atoms is stereospecifically released as water.[1] The absolute stereochemistry for this novel oxidation reaction, which may be envisaged to occur *via* the intermediacy of a *gem* diol of



SCHEME 1. i, 2-step reaction involving placental microsomes + NADP.

the type (**3**), has been elucidated using two independent approaches.[3,4] It is also known that in the further conversion of the 19-aldehyde into oestrogen, the C-19 is removed as formic acid and once again the reaction compulsorily requires NADPH and molecular oxygen.[1] In order to rationalise the requirements of NADPH and O_2 in the reaction (**4**) → (**5**) two types of mechanism have been considered. The first mechanism assumes the involvement of yet another hydroxylation step in the pathway to give the 1β-hydroxy intermediate (**7**), which on subsequent elimination and rearrangement forms the aromatic ring present in oestrogen (**5**) [mechanism (A) (Scheme 2)]. In the second mechanism, NADPH and O_2 are believed to be involved in an epoxidation reaction,[5] to give the epoxy ketone (**8**). The conversion of (**8**) into oestrogen may occur by the abbreviated sequence (**4**) → (**5**) [mechanism (B)]. We now describe experiments showing that the conversion of a 19-aldehyde precursor into oestrogen in the presence of ^{18}O gas is attended by the incorporation of the isotopic oxygen into formate. This result highlights the participation of a new mechanism for the C-10–C-19 bond cleavage step.

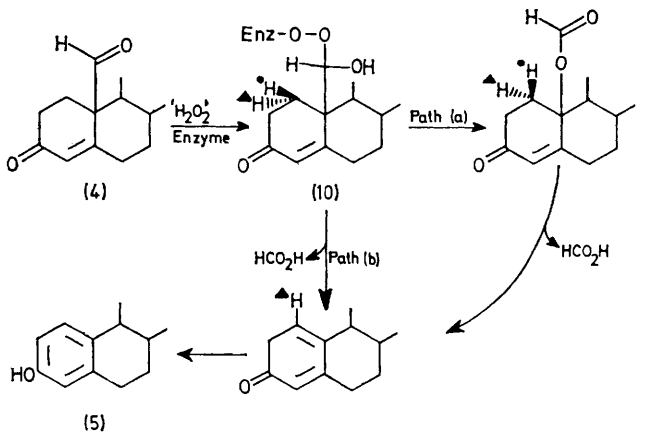
Attention is drawn to the fact that the penultimate intermediate (**4**) in the oestrogen biosynthesis pathway may be generated *in situ*[1] from the conveniently available Δ^5-3β-hydroxy-19-aldehyde (**6**) through a two-step enzymic reaction. The aerobic incubation of [19-tritiated]Δ^5-3β-hydroxy-19-aldehyde (**6**) (500 μg) with placental microsomes, in the presence of NADP and NADPH, routinely gave [^{3}H]formic acid (50 μg), representing a 70% radiochemical yield. The [19-tritiated] Δ^5-3β-hydroxy-19-aldehyde (**6**) was now incubated as above, but in the presence of oxygen containing 20·4 atom % excess of ^{18}O (hereafter referred to as the experimental incubation). The formate produced in this experiment was converted into benzyl formate and analysed by g.l.c., which showed that 40 μg of formate had been produced during the biosynthesis, which is of the same order as the radiochemical determination. Mass spectrometric analysis of the benzyl formate showed a molecular ion peak at *m/e* 136, corresponding to all the ^{16}O species, and a further peak at *m/e* 138, corresponding to the ^{18}O species. A comparison of the peak heights of these two molecular ions showed that the benzyl formate, originating from the experimental incubation, contained 14·6 atom % excess ^{18}O. Controls showed that, in incubations under $^{18}O_2$ gas in the absence of substrate, the amount of formate formed as estimated by g.l.c. was only one fifth of that produced in the experimental incubation and contained no



SCHEME 2. Mechanisms rationalising the involvement of NADPH and O_2.

significant amount of ^{18}O. A further control excluded the possibility of exchange reactions between formic acid and $^{18}O_2$ gas.

The cumulative results presented above exclude mechanism (B) (Scheme 2) for the C-10–C-19 bond cleavage step in oestrogen biosynthesis. The possibility that NADPH and O_2 are required to produce the 1β-hydroxy intermediate (**7**), which then undergoes an electrocyclic rearrangement, *via* a



SCHEME 3. Path (a): *via* Baeyer–Villiger process. Path (b): elimination *via* a concerted process.

four-membered transition state,† to give the 9-10 double bond of oestrogen, although not directly excluded by the experiment detailed above, is unlikely on stereochemical grounds. In our view the ^{18}O results are best rationalized by assuming that the final stage in oestrogen biosynthesis requires the participation of a peroxide, formed from NADPH and O_2, which participates in the reaction with the carbonyl group of the aldehyde, giving an intermediary complex [represented by a hypothetical structure of the type (**10**), Scheme 3]. The latter species may then produce oestrogen either *via* a Baeyer–Villiger process (path a) in two steps, or rearrange directly through a cyclic mechanism (path b).

In view of the fact that the enzymological and chemical features of the removal of C-19 [Scheme 1(a)] in oestrogen biosynthesis and C-32 [Scheme 1(b)] in sterol biosynthesis[6] are similar, it is possible that the cleavage of the C-14–C-32 bond in sterol biosynthesis may also occur through the pathway suggested above for oestrogen biosynthesis.

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† The consideration of such a process stems from the fact that it is the 1β-hydrogen atom which is lost in the aromatization reaction (T. Morato, K. Raab, H. J. Brodie, M. Hayano, and R. I. Dorfman, *J. Amer. Chem. Soc.*, 1962, **84**, 3764).

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