Oxidative Bonding of Natural Oestrogens to DNA by Chemical and Metabolic Means

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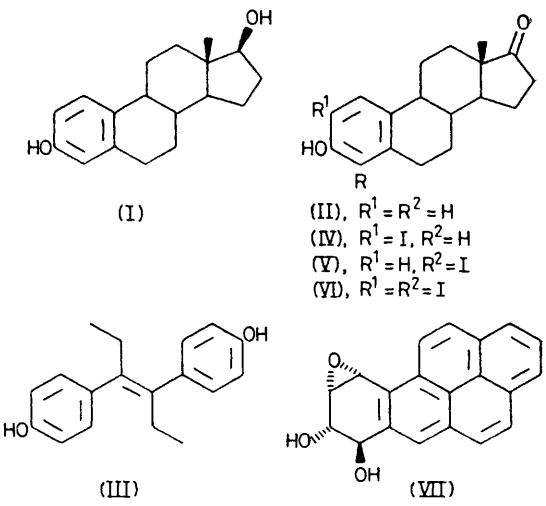
Summary Oestradiol and oestrone can be covalently bonded to DNA as a result of oxidation by iodine or hydrogen peroxide and, alternatively, by incubation with a rat liver microsomal preparation *in vitro*: radioisotope studies show that these processes are mechanistically distinct.

THERE is growing causal evidence associating cancerous changes in hormone-sensitive[1] and hormone-insensitive[2] tissues with oestrogenic compounds.[3] Oestradiol (I) and oestrone (II) have not been identified as mutagens in the Ames' test[4] but they are known to form physical complexes with DNA.[5] The synthetic oestrogen, diethylstilboestrol (III), is an established carcinogen[6] and can be bound covalently to DNA by chemical oxidation[7] or by enzymic processes *in vitro* and *in vivo*.[8]

Oestrogenic steroids are metabolised[9] by annular hydroxylation at positions-2, -6, -7, and -16 under conditions which can also lead to covalent binding of 2-hydroxyoestrogenic steroids to microsomal protein. A mechanism has been proposed for this process which involves the superoxide anion radical in the formation of a reactive metabolic intermediate.[10] We now report results on the covalent binding of oestradiol and oestrone to DNA by chemical methods and also by the use of rat liver microsomal preparations.



(I)

(II), $R^1 = R^2 = H$
(IV), $R^1 = I$, $R^2 = H$
(V), $R^1 = H$, $R^2 = I$
(VI), $R^1 = R^2 = I$

(III) (VII)

[4-^{14}C]Oestradiol and [6,7(*n*)-^{3}H]oestradiol were co-purified by t.l.c. and converted into a physical complex with calf thymus DNA as previously described for diethylstilboestrol.[7] Aliquot portions were treated with iodine (concentrations up to 10^{-2} M) for 3 days at 20 °C and the DNA which then precipitated was washed to remove

unbound steroid, hydrolysed with 4N HCl, and the residual radioactivity determined by liquid scintillation counting. At the highest concentration of iodine used, the level of covalent binding of oestradiol to DNA, based on ^{14}C activities, was $1{\cdot}5 \times 10^{-4}$ mol/mol DNA base pairs. These experiments were repeated using [4-^{14}C]oestrone and [2,4,6,7(*n*)-^{3}H]oestrone which gave an oestrone binding level of $1{\cdot}2 \times 10^{-4}$ mol/mol DNA base pairs.

In the case of oestradiol, the tritium : carbon-14 ratio in the covalent complexes was essentially independent of the level of steroidal binding and the same as that of the physical complex. However, the $^{3}H:^{14}C$ ratio of the oestrone covalent complex showed a loss of 40% of the tritium relative to the initial physical complex.

Direct iodination[11] of oestrone (II) gave a mixture of the 2- and 4-iodo-oestrones in approximately equal amounts. These were separated by h.p.l.c. and identified by n.m.r. spectroscopy; (IV), $J_{1,4} \not> 0{\cdot}5$ Hz and (V), $J_{1,2} = 8$ Hz at 220 MHz. Since preliminary results indicate that the formation of (IV) and (V) from (II) is associated with a sizeable primary kinetic hydrogen isotope effect, doubly labelled (II) was completely converted into the 2,4-di-iodo-oestrone (VI) with the loss of some 60% of its initial tritium content.

The use of hydrogen peroxide (30 mM) in place of iodine led to similar overall levels of binding of both steroids to DNA, again with no loss of tritium in the case of oestradiol but now with $<10\%$ loss of tritium in the case of oestrone.

The two oestrogens were also bound covalently to DNA during incubation with washed rat liver microsomal preparations in the presence of NADPH and oxygen. This cytochrome P450 system, used as described elsewhere,[8] effected a level of binding of oestradiol of 10^{-4} mol/mol base pairs without loss of tritium and of oestrone of 3×10^{-5} mol/mol base pairs with only some 5% loss of tritium.

These data show that binding of oestrogens to DNA by iodine oxidation is accompanied by displacement of hydrogen from either position-2 or -4 though there is apparently[12] no loss from positions-6 and -7. While the small but significant loss of tritium from ring A during hydrogen peroxide-mediated binding is difficult to interpret, the effectively complete retention of tritium in the microsomal binding experiments restricts the number of possible mechanisms for this process. Two deserve special comment.

First, Breuer and his colleagues[13] have shown that rat liver tissue contains a steroid epoxidase which can operate on a Δ^{16}-oestratetraen-3-ol and which might thus cause oestrone to bind to DNA at position-16. Secondly, the hydroxylation of oestrogens at position-2 may reasonably involve formation of the 1,2-α-epoxide as a reactive metabolic intermediate which would be capable of alkylating DNA and protein. Such a process would have the same regiospecificity and the same absolute stereospecificity as that by which benzo[*a*]pyrene is bound to RNA *in vivo*[14,15] *via* the metabolite (VII). It is probable that this hydroxylating system in rat liver microsomes operates on steroids as its normal substrates[9] and only adventitiously on polycyclic aromatic hydrocarbons.

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