Binding of 7,12-Dimethylbenz[*a*]anthracene to DNA Investigated by Tritium Displacement

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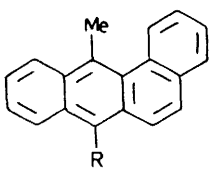
Summary 7,12-Dimethylbenz[*a*]anthracene, specifically labelled with tritium in the 7-methyl group, has been prepared and then bound covalently to DNA both photochemically *in vitro* and metabolically *in vivo*; a significant proportion of the tritium is displaced in both cases.

7,12-DIMETHYLBENZ[*a*]ANTHRACENE (**1**) is one of the most carcinogenic aromatic hydrocarbons and binds covalently to DNA *in vivo*[1] and also by u.v. irradiation[2] or oxidation by hydrogen peroxide[3] of physical complexes of the hydrocarbon and DNA *in vitro*. The position of reaction on the hydrocarbon is not known although evidence has been presented recently[4] in support of metabolic activation of benz[*a*]anthracene by epoxidation at positions 10 and 11. This problem should be resolvable by evaluation of the displacement of hydrogen associated with the binding of the hydrocarbon to DNA[5] in conjunction with specific syntheses of isotopically substituted hydrocarbons. We now report the results of such a study for 7,12-dimethylbenz[*a*]anthracene.



(1); R = Me
(2); R = CH_2I
(3); R = CH_2T
(4); R = CH_2OH
(5); R = $CH_2^{\bullet}$

7-Iodomethyl-12-methylbenz[*a*]anthracene[6] (**2**) was reduced with tritiated sodium borohydride in dioxan–dimethylsulphoxide solution[7] to give [7-methyl-^{3}H]-7,12-dimethylbenz[*a*]anthracene (**3**) in 52% yield after chromatographic purification. This hydrocarbon was mixed with 7,12-dimethyl[12-^{14}C]benz[*a*]anthracene and carrier hydrocarbon (**1**) and the mixture repurified by t.l.c. on silica gel. A portion of this material was analysed for ^{3}H and ^{14}C by liquid scintillation spectrometry.

A portion of the doubly-labelled hydrocarbon was used to prepare a physical complex with calf thymus DNA which was then subjected to long wavelength u.v. irradiation.[2] The covalent DNA–hydrocarbon complex thus formed was analysed for both isotopes and showed a retention of 72 ± 5% of the tritium present in the initial hydrocarbon sample. A similar experiment using generally tritiated (**1**) showed that the loss of tritium was essentially independent of the duration of irradiation and of the amount of hydrocarbon bound to DNA. A second portion of the same sample of specifically doubly-labelled (**1**) was bound oxidatively to calf thymus DNA using hydrogen peroxide as described by Morreal.[3] In this case the covalent DNA–hydrocarbon product showed no significant loss of tritium. Finally, a further portion of the doubly-labelled (**1**) was incubated with a Vero (monkey kidney) continuous cell culture and the DNA isolated and analysed by gel-filtration and by alkaline sucrose gradient ultracentrifugation.[5] The DNA–hydrocarbon fraction thus characterised showed a net retention of tritium of 74 ± 6% relative to the parent hydrocarbon sample.

These results demonstrate that the binding of (**1**) to DNA both metabolically *in vivo* and photochemically *in vitro* is associated with chemical reaction at the 7-methyl group and results in the displacement of 26—28% of its tritium content. This loss is most simply accounted for by displacement of a single hydrogen in conjunction with a very small primary kinetic isotope effect (*ca.* 1·2) but could possibly result from the displacement of two hydrogens with a larger kinetic isotope effect (*ca.* 3·0). The well known metabolism of (**1**) to 7-hydroxy-methyl-12-methylbenz[*a*]anthracene[8] (**4**) supports the former interpretation. We therefore suggest that the hydrocarbon (**1**) may bind to DNA as a result of photochemical or metabolic oxidation at the 7-methyl group, possibly to give the benzyl radical (**5**). Such a radical would be readily oxidised further to the alcohol (**4**) in the metabolic detoxification† of (**1**).

The fact that hydrogen peroxide effects binding of (**1**) to DNA without material loss of tritium suggests a different mechanism for such binding.‡ It thereby provides the first experimental indication that hydrocarbon linkage to DNA under metabolic conditions may proceed by the simultaneous operation of different processes for activation of aromatic hydrocarbons.

This work was supported in part by the Medical Research Council, by the Yorkshire Cancer Research Campaign, and by the provision of an S.R.C. Research Studentship (to A.J.F.). We thank Dr. C. W. Potter for assistance with tissue culture facilities.

(*Received*, 25*th February* 1975; *Com.* 241.)

† An alternative, more elaborate interpretation is that photochemical and metabolic activation of the 7-methyl group is an event which precedes the covalent binding of DNA to (**1**) at a different position on the hydrocarbon, and is under investigation.

‡ The possible operation of the same mechanism as in the other two cases would involve a primary kinetic isotope effect of at least tenfold.

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