Displacement of Tritium from 6-Tritiobenzo[*a*]pyrene on Covalent Binding to DNA

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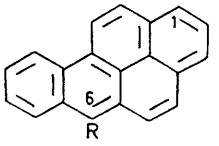
Summary 6-Tritiobenzo[*a*]pyrene has been prepared from 6-iodobenzo[*a*]pyrene *via* lithiation and reaction with tritiated water: photochemical binding of this material to DNA resulted in the loss of 92% of its tritium content while binding using rat liver microsomes showed a loss of only 20% of tritium.

THE covalent binding of polycyclic aromatic hydrocarbons to DNA is strongly associated with their carcinogenic behaviour.[1] This appears to be a side effect of their oxidation *in vivo* by metabolic processes, whose prime function is one of detoxification, for which there are two principle, competing pathways. One proceeds by epoxidation[2] at one or more double bonds and normally leads to a variety of dihydrodiols. The other results in the production of quinones arising from hydroxylation at C-6 at an early stage.[3] The relative importance of these processes varies not only between different mammalian species but also from one individual to another.[4]

In principle, the relative contributions made by these types of oxidation to the covalent binding of aromatic hydrocarbons to DNA can be examined as a function of the displacement of tritium from various annular positions.[5] This technique has been adopted for benzo[*a*]pyrene generally labelled with tritium. When this material, in which 34% of the tritium content is located[6,7] at position-6, is bound photochemically to DNA there is a 30% displacement of tritium[6] which is commensurate with binding at position-6. On the other hand, binding *in vitro* by microsomal preparations or in mouse embryo cells in tissue culture has afforded hydrolysis products with only 5—10% loss of tritium.[7] It is apparent that this difference should be resolved by comparable studies using a specifically labelled

benzo[*a*]pyrene and we report here our results using 6-tritiobenzo[*a*]pyrene (II).



(I) R = ^{1}H
(II) R = ^{3}H
(III) R = I
(IV) R = ^{2}H

6-Iodobenzo[*a*]pyrene[8] (III) was treated with butyllithium at −78 °C, shortly followed by 1 equiv. of tritiated water[9] to give 6-tritiobenzo[*a*]pyrene (II) of specific activity 28 mCi mmol^{-1} (70% yield). This preparation is comparable to the Grignard route used by Warshawsky and Calvin[8] but, in our hands, proved to be experimentally less exacting. Samples of 6-bromo- and 6-nitro-benzo[*a*]pyrene prepared from this material showed that at least 90% of its tritium content is located at C-6. 6-Deuteriobenzo[*a*]pyrene (IV) was prepared in a similar fashion using D_2O and gave a 100 MHz n.m.r. spectrum as for (I) except for the loss of the singlet at τ 1·58 for H-6.

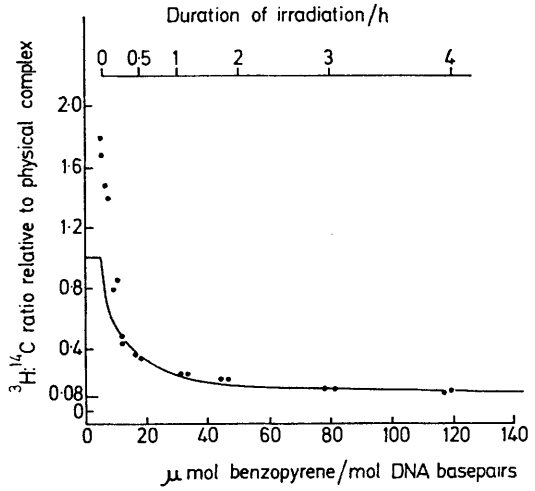


FIGURE. Ratio of ^{3}H:^{14}C incorporated into native calf thymus DNA using doubly labelled benzo[*a*]pyrene as a function of total hydrocarbon incorporation including dark binding. The theoretical curve is calculated for zero loss of tritium on dark binding and 92% loss of tritium for total photochemical binding.

A sample of this 6-tritiobenzo[*a*]pyrene was admixed with [7,10-^{14}C]benzo[*a*]pyrene and carrier hydrocarbon, purified by t.l.c. and bound photochemically to calf thymus DNA as described previously.[5] Aliquot portions were irradiated for intervals up to 4 h and subjected to complete hydrolysis by 4 N HCl prior to determination of radioactivity. The net binding of hydrocarbon to DNA was calculated from the carbon-14 incorporation data.

The results (Figure) show three clear features. First, photochemical bonding of (II) to DNA increases continuously with duration of irradiation for at least 4 h and is associated with a limiting retention of 8% of tritium relative to that present in the initial DNA–hydrocarbon physical complex. Secondly, some 5 μmol of hydrocarbon per mol of DNA base-pairs remains associated with DNA in unirradiated samples and suffers no loss of tritium. Thirdly, at the lowest levels of hydrocarbon association with DNA, corresponding to less than 150 d.p.m. of carbon-14, the ^{3}H:^{14}C ratio considerably exceeds that of the initial physical complex.

The same doubly labelled benzo[*a*]pyrene was bound to DNA by incubation with calf thymus DNA, NADPH, and a microsomal preparation from the liver of rats pretreated with 3-methylcholanthrene.[10] The DNA was extracted by the phenol technique[10] and shown to have incorporated 150 μmol (I) per mol base-pair. Hydrolysis of this material and radiochemical assay showed retention of 80 ± 2% of tritium.

The data on the photochemical binding show that the ^{3}H:^{14}C ratio method provides consistent results at high levels of hydrocarbon binding to DNA but may give an overestimate of tritium uptake at low carbon-14 incorporation levels. They establish unambiguously that photochemical binding of DNA with (I) involves attachement at position-6 of benzo[*a*]pyrene.† On the other hand, microsomal binding of (II) to DNA is associated with the loss of only 20% of its tritium content. This result thereby provides the first clear indication that metabolic binding of benzo[*a*]pyrene to DNA is likely to proceed simultaneously by at least two processes.

It has been estimated by Ts'o that some 20% of total benzo[*a*]pyrene metabolism in rat liver involves the hydroxylation of (I) at position-6. The present data correlate well with that proposal and suggest that such a process, involving loss of tritium from (II), is a minor route for the metabolic binding of benzo[*a*]pyrene to DNA and that a major route proceeds *via* metabolites which do not involve a substitution process at position-6.

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† Unchanged hydrocarbon recovered at the completion of photochemical binding showed no change in the specific activity of tritium.

[1] P. Brookes and P. D. Lawley, *Nature*, 1964, **202**, 781.
[2] A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, and I. Y. Wang, *J. Medicin. Chem.*, 1973, **16**, 502; P. Sims, P. L. Grover, A. Swaisland, K. Pal, and A. Hewer, *Nature*, 1974, **252**, 326.
[3] (a) C. Nagata, M. Inomata, M. Kodama, and Y. Tagashira, *Gann*, 1968, **59**, 289; (b) S. Lesko, W. Caspary, R. Lorentzen, and P. O. P. Ts'o, *Biochemistry*, 1975, **14**, 3978.
[4] C. H. Fox, J. K. Selkirk, F. M. Price, R. G. Croy, K. K. Sanford, and M. Cottler-Fox, *Cancer Res.*, 1975, **35**, 3551.
[5] G. M. Blackburn, J. Buckingham, R. G. Fenwick, P. Taussig, and M. H. Thompson, *J.C.S. Perkin I*, 1973, 2809.
[6] G. M. Blackburn, P. E. Taussig, and J. P. Will, *J.C.S. Chem. Comm.*, 1974, 907.
[7] M. R. Osborne, M. H. Thompson, H. W. S. King, and P. Brookes, *Internat. J. Cancer*, 1975, **16**, 659.
[8] D. Warshawsky and M. Calvin, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 541.
[9] *Cf.* A. Streitwieser, R. G. Lawler, and S. Schwaab, *J. Org. Chem.*, 1965, **30**, 1470.
[10] H. V. Gelboin, *Cancer Res.*, 1969, **29**, 1272.