Binding of Benzo[*a*]pyrene to DNA Investigated by Tritium Displacement

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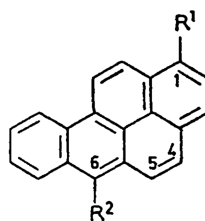
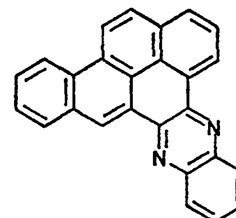
Summary The distribution of tritium in generally-labelled benzo[*a*]pyrene is determined for positions 1, 4–5, and 6 by transformation into its 1-acetyl-,[4,5-*b*]quinoxalino-, and 6-nitro-derivatives; the extent of loss of tritium from the same hydrocarbon on binding to DNA either photochemically *in vitro* or metabolically *in vivo* shows that these processes are not associated with the *K*-region of the hydrocarbon, but are most likely to result from covalent substitution at position 6 or 1.

The carcinogenicity of various polycyclic aromatic hydrocarbons has been shown to correlate with their binding to nucleic acids *in vivo*.[1] Such binding can also be achieved *in vitro* by photochemical[2] or oxidative[3] methods. The photochemical binding of tritiated anthracene, benzo[*a*]-pyrene, and 3-methylcholanthrene to DNA is known to be attended by a reduction in the tritium content of the hydrocarbons.[4] This observation suggests a means of identifying the positions on these hydrocarbons to which the nucleic acid is bonded and this report describes results of this approach for the binding of benzo[*a*]pyrene to DNA *in vitro* and *in vivo*.

Generally tritiated benzo[*a*]pyrene was obtained using the catalytic exchange method[5] and admixed with [3,6-^{14}C]benzo[*a*]pyrene and carrier, the hydrocarbon (I). The ratio of the specific activities of tritium and carbon-14 provided a reference standard for the following experiments.

1-Acetylbenzo[*a*]pyrene (II) was prepared by direct acetylation[6] of the hydrocarbon. The pure product had a ^{3}H/^{14}C ratio of 72% relative to (I). Unchanged hydrocarbon was recovered from the reaction mixture after 95% completion of the acetylation and showed an enrichment of tritium corresponding to a small kinetic isotope effect, $k_H/k_T = 1{\cdot}13$. 6-Nitrobenzo[*a*]pyrene[7] was obtained by nitration of (I) and gave a ^{3}H/^{14}C ratio of 66% relative to (I). Recovered hydrocarbon after near-complete reaction showed a very small enrichment of tritium which corresponded to a kinetic isotope effect not significantly greater than unity. The same sample of (I) was oxidised by osmium tetroxide followed by chromic acid to give benzo[*a*]-pyrene-4,5-quinone which was characterised and purified as the quinoxaline[8] (IV). The ^{3}H/^{14}C ratio for this material was not less than 95% that of (I).





(I) R^1 = R^2 = H
(II) R^1 = MeCO; R^2 = H
(III) R^1 = H; R^2 = NO$_2$

(IV)

These results show that the distribution of tritium *in this sample* of benzo[*a*]pyrene is 34% in position-6, 28% in position-1, and not more than 5% jointly in positions-4 and -5. This order of substitution by catalytic tritio-deprotonation follows qualitatively the predicted reactivities of these positions calculated by Dewar.[9]

A physical complex of the same tritiated sample was irradiated for 10 h with u.v. light and the covalently bound DNA–benzo[*a*]pyrene complex purified.[4] This material showed a $^{3}H/^{14}C$ ratio of 69% relative to that of the DNA–hydrocarbon physical complex. Finally, the doubly labelled benzo[*a*]pyrene was incubated with mouse kidney cells in tissue culture in the dark and a sample of nuclear DNA obtained by cell lysis, alkaline sucrose gradient centrifugation, and gel-filtration. The resulting high-molecular-weight DNA showed a $^{3}H/^{14}C$ ratio of 65% of that for the hydrocarbon in the initial culture medium.

The magnitude of displacement of tritium which occurs on binding benzo[*a*]pyrene to DNA *in vitro* (31%) and *in vivo* (35%) is thus very much greater than the combined tritium content of positions-4 and -5 in the parent hydrocarbon. Therefore, the *K*-region of benzo[*a*]pyrene must be rejected as a binding site for DNA as variously suggested for photochemical addition and for metabolic processes involving oxidation to a **4**,5-epoxide.

These results do not permit an unambiguous assignment of the position on the hydrocarbon which is involved in binding to DNA but the close correspondence between the tritium content of position-6 in (I) and that lost on binding to DNA is to be compared both with the established photochemical linkage[10] of benzo[*a*]pyrene to 1-methylcytosine and to thymine at position-6 and with its anodic coupling to pyridine at the same site.[11]

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