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6-AZIDOBENZO[a]PYRENE.
A PHOTOACTIVE DERIVATIVE OF BENZO[a]PYRENE FOR PHOTOAFFINITY LABELING

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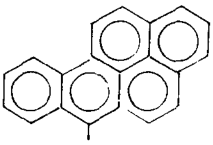
Summary: The synthesis, photochemical break-down and photobinding to DNA *in vitro* of 6-azidobenzo[a]pyrene are described.

Benzo[a]pyrene is a potent mutagen and carcinogen, whose mode of action is being intensively investigated.[1] It is known that BaP undergoes conversion *in vivo*, *via* cytochrome P-450 mixed function oxygenases to metabolically active forms, *e.g.*, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene.[2] This material can undergo covalent binding to DNA, RNA and protein. Benzo[a]pyrene can also undergo non-covalent interactions with DNA, primarily *via* intercalation.[3]

In a study of the intercalation sites of BaP in DNA and chromatin, and the interaction of BaP with the mixed function oxygenase by photoaffinity labeling,[4] we have synthesized a photoactive benzo[a]pyrene derivative, *i.e.*, 6-azidobenzo[a]pyrene (6-N_3-BaP) (Scheme).



(I)

x = a: H
b: NO_2
c: NH_2
d: N_3

$$\mathrm{Ia} \xrightarrow{HNO_3} \mathrm{Ib} \xrightarrow[Pd/C]{H_2NNH_2}$$

$$\mathrm{Ic} \xrightarrow[2.\,TsN_3]{1.\,BuLi} \mathrm{Id}$$

Benzo[a]pyrene (Ia) was converted to 6-NO_2-BaP (Ib),[5] which was reduced with hydrazine (Pd/C, EtOH).[6] The 6-NH_2-BaP (Ic) was treated with an excess of n-BuLi in dry THF under N_2 (until the red color persisted), and then with an equimolar amount of p-toluenesulfonyl azide. 6-N_3-BaP (Id) (30%)[7] and 6-NH_2-BaP (60%) were isolated by TLC (silica gel, eluted once with benzene, Rf, 0.6 and 0.2).

Formation of the azide *via* the diazonium salt was not possible due to the ease by which the latter was converted to the 1,6-quinone.

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The photochemistry of 6-N_3-BaP resembles that of 1-N_3-pyrene,[8] giving the amine upon irradiation (15% in methanol saturated with oxygen from ambient air, and 30% in degassed methanol); the half-life of the triplet nitrene was measured by flash photolysis as 0.85 msec.

On mixing with a solution of calf thymus DNA, the absorption spectrum of 6-N_3-BaP was shifted ca. 18 nm towards longer wavelengths, suggesting that an intercalation had taken place. Preliminary experiments with ^{3}H-labeled 6-N_3-BaP[9] indicate that on photolysis more than 40% of the photo-label becomes covalently bound to DNA. The products formed in this reaction are currently under investigation. The high efficiency of binding to DNA indicates that azide substituted polycyclic aromatic hydrocarbons may be generally useful for studying intercalation sites in DNA, and for active site labeling in P-450 oxygenases.

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6. Yield 65%, mp. 238-240°C (dec). Litt. 237-239°C (dec).[5]
7. IR (KBr, cm^{-1}): 2100 (-N_3), UV_{max} (EtOH, nm): 291, 306, 360, 379, mp. 105°C dec. ms (m/e): 293 (M^+ 7%), 267 (100%), 239 (33%). The 267 peak probably arises from decomposition of the azide on the inlet probe.
8. Takahiro, T., Tsuguo, Y. and Michio, T. Nippon Yakugaku Kaishi 12, 2074 (1975).
9. ^{3}H-N_3-BaP was prepared from ^{3}H-NH_2-BaP which was made by a tritium exchange with ^3H_2O (20 mCi/μl) in conc. H_2SO_4 (Warshawsky, D. and Calvin, M. Biochem. Biophys. Res. Commun. 63, 541 (1975). A specific activity of ca. 40 mCi/mmol was obtained.

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