Reactions of Phosphodiesters with Epoxides of Polycyclic Aromatic Hydrocarbons

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(±)-*t*-9, *t*-10-Epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-*r*-7, *t*-8-diol and other polycyclic aromatic hydrocarbon epoxides react with phosphodiesters to give phosphotriesters by regiospecific and stereospecific opening of the epoxide.

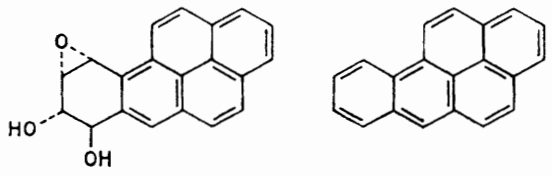
Polycyclic aromatic hydrocarbons (PAH) are considered to be the most prevalent environmental carcinogens.[1] Recent evidence suggests that these PAH are first metabolized to reactive epoxides and that it is the reaction of these PAH epoxides with cellular biomolecules such as DNA that may be the starting point of carcinogenesis.[2] Results from several laboratories have established[3] (+)-*t*-9,*t*-10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-*r*-7,*t*-8-diol (BPDE, **1**) as the active metabolite of the carcinogenic hydrocarbon benzo[*a*]pyrene (BP, **2**). Reactions of BPDE with nucleic acids have also been studied[4] and the principal covalent adducts appear to be derived from the opening of the epoxide by the NH_2 group of guanosine and other DNA bases (*N*-alkylation).[5] The possibility that these epoxides can also react with the phosphate groups (*P*-alkylation) of nucleic acids to give phosphotriesters has been considered.[6] However, no direct evidence for such a reaction has been obtained. Specifically, (±)-BPDE was reported *not* to give phosphotriester adducts[7] on reaction with dibutyl phosphate.

We report that PAH epoxides do react with phosphodiesters to give triesters. Furthermore, contrary to the previous report,[7] (±)-BPDE reacts cleanly with dialkyl phosphates to give phosphotriester adducts in a regio- and stereo-specific fashion.

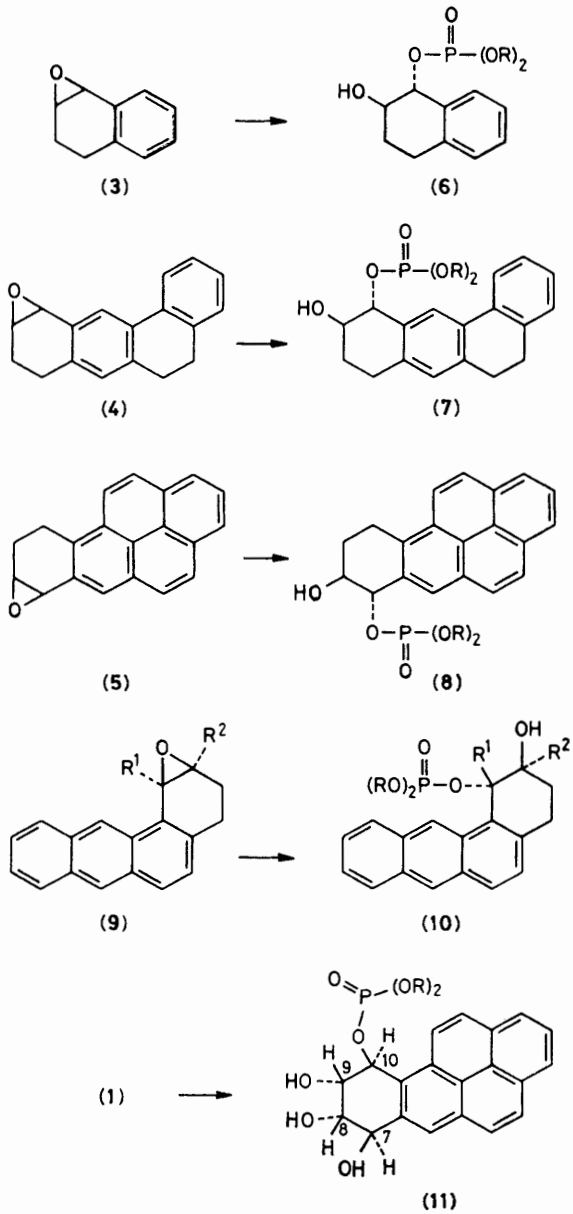
PAH epoxides such as (**3**), (**4**), and (**5**) react with dibenzyl or diethyl phosphates in benzene to give the corresponding adducts (**6**), (**7**), and (**8**) in good yield (*ca.* 80%). In all cases, the attachment of the phosphate group is at the benzylic position and only one stereoisomer is formed. Based on ^1H n.m.r. spectroscopy, a *trans*-stereochemistry is assigned (Scheme 1, *vide infra*), suggesting a S_N2 type opening of the epoxide.[8]

1,2,3,4-Tetrahydro-1,2-epoxybenz[*a*]anthracene (**9**, R^1 = R^2 = H), a bay-region epoxide, reacts with dibenzyl or diethyl phosphate in benzene to give the adduct (**10**, R^1 = R^2 = H). To confirm the position of attachment of the phosphate group, two deuteriated compounds were synthesized with deuterium at the benzylic (**9**, R^1 = D, R^2 = H) and the homobenzylic (**9**, R^1 = H, R^2 = D) positions. By comparing the ^1H n.m.r. spectra of the three adducts formed, it is possible to establish unequivocally that the phosphate opens the epoxide ring at the benzylic position indicated in Scheme 1. Confirmatory evidence was obtained from ^{31}P n.m.r. spectroscopy of the adducts by noting the multiplicities of the ^{31}P signals.

anti-BPDE (**1**) reacts with dibenzyl phosphate in [$^2H_8$]-tetrahydrofuran (THF) at room temperature to give the adduct (**11a**) exclusively. The structure of (**11a**) was deduced from its ^1H n.m.r. spectrum (Figure 1). The signal at 6.50 is assigned to 10-H which is a doublet of doublets due to coupling with 9-H as well as with phosphorus. Other assignments are indicated in Figure 1 and are confirmed by decoupling experiments. The reaction of (**1**) with diethyl phosphate in [$^2H_8$]THF is considerably slower. Nevertheless, the adduct (**11b**) is obtained, as shown by the appearance of the 10-H dd signal at 6.45.



(1) (2)



(3) (6) (4) (7) (5) (8) (9) (10) (1) (11)

a; R = $PhCH_2$
b; R = $MeCH_2$

Scheme 1

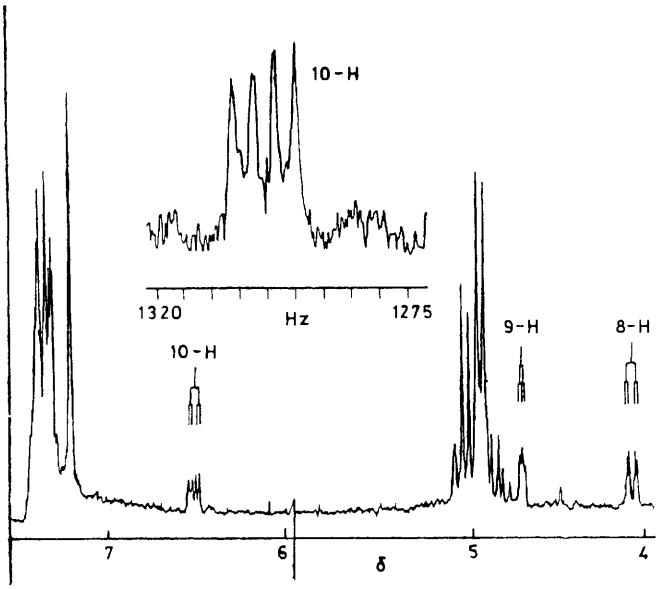


Figure 1. 200 MHz ^{1}H n.m.r. spectrum of (**11a**) in [$^2H_8$]THF. The inset shows an expansion of the δ 6.5 region.

Table 1. Coupling constants (and chemical shifts) of the benzylic methine proton with its vicinal hydrogen atom ($CDCl_3$) for the phosphotriester adducts.

PAH epoxide	Phospho-triester adduct	J/Hz (δ) **a**; R = $PhCH_2$	**b**; R = $MeCH_2$
(**3**)	(**6**)	6.8 (5.38)	6.6 (5.2)
(**4**)	(**7**)	7.4 (5.5)	7.0 (5.4)
(**5**)	(**8**)	7.1 (5.6)	7.2 (5.7)
(**9**)	(**10**)	4.5 (6.3)	3.2 (6.4)
(**1**)	(**11**)	3.6 (6.5)[a]	3.8(6.45)[a]

[a] Measured in [$^2H_8$]THF.

From ^{1}H n.m.r. studies of the various phosphotriester adducts obtained, the benzylic proton in (**6**), (**7**), or (**8**) has a vicinal coupling constant of *ca.* 7 Hz, whereas in the adducts (**10**) or (**11**) derived from bay-region epoxides, the vicinal coupling constant is *ca.* 3 Hz (Table 1). This suggests that the adducts (**10**) and (**11**) exist mainly in the quasi-diaxial conformation. A similar conclusion has been reached for other adducts (*e.g.* tetraols or thiol adducts) of bay-region epoxides from n.m.r. studies and *X*-ray determinations.[9]

The present results contrast sharply with the reactions of K-region arene oxides with phosphodiesters to give phenols.[10] K-region epoxides are sometimes found to be the major metabolites of PAH. They are also effective electrophiles in their reaction with a number of nucleophiles such as sulphides,[11] amines,[12,13] and hydroxides.[12] However, K-region epoxides appear to be weak carcinogens and no *in vivo* adduct of a K-region epoxide with DNA has been observed so far. The distinct pathways encountered in the reaction of phosphodiesters with these two types of metabolites of PAH may be one of the many chemical factors which should be taken into consideration when trying to understand the carcinogenesis of PAH.

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