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REACTION OF CYCLOHEXENE OXIDES WITH PHOSPHODIESTERS - TOWARDS UNDERSTANDING THE REACTION OF BENZO[a]PYRENE DIOL EPOXIDE WITH DNA

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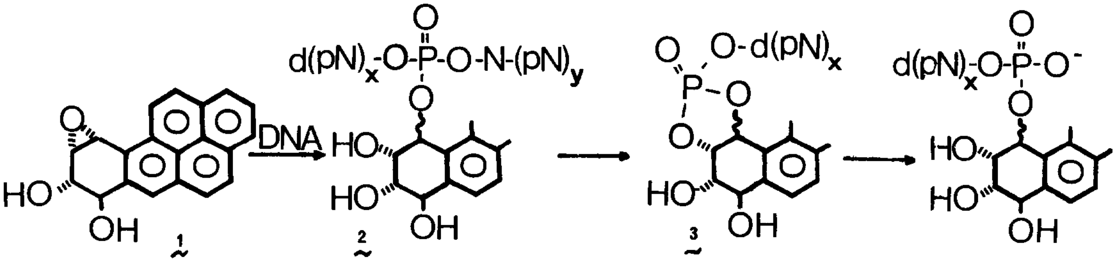
Phosphodiesters react with cyclohexene oxides to give phosphotriesters with high stereo- and regiospecificity.

Benzo[a]pyrene (BP), a member of polycyclic aromatic hydrocarbons (PAH), is considered to be one of the most prevalent environmental carcinogens[1,2]. Recent evidence suggests that BP is first metabolized in vivo to 7β, 8α-dihydroxy - 9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (1 BP diol epoxide)[3]. There is also strong evidence to indicate that it is the binding of cellular deoxyribonucleic acid (DNA) with this metabolite of BP which is the primary event leading to carcinogenesis[4]. Recently, Calvin et al[5] found that BP diol epoxide 1 elicits a concentration - dependent strand scission of superhelical Col El DNA in an in vitro reaction. The fragmentation of DNA is not attributed to a mechanism involving alkylation of the pyrimidine or purine bases[6]. This is based on the kinetics difference between 1 and dimethylsulfate on the rate of strand scission on DNA. Calvin et al suggest that the formation of a phosphotriester 2 followed by a facile hydrolysis of the cyclic intermediate 3 is the most likely explanation for the rapid nicking of DNA by diol epoxide (scheme1)[6] If confirmed, the damage to cellular DNA may account for the high mutagenic and potentially carcinogenic activity of BP diol epoxide. Calvin's scheme has however not been supported by chemical evidence.

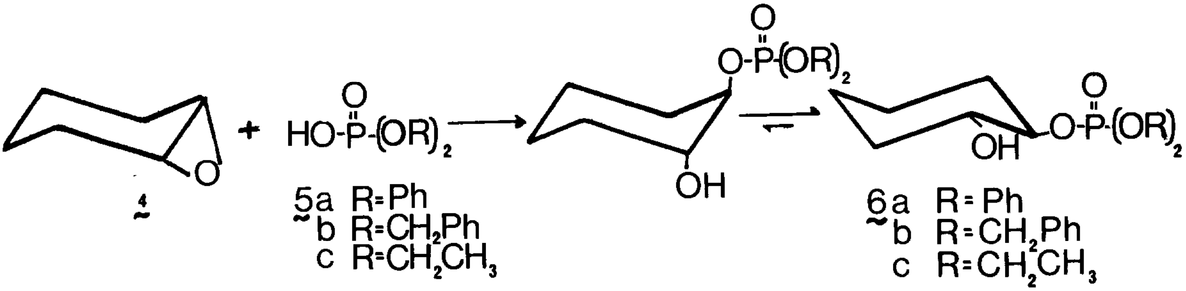


scheme 1

We have examined the reaction of cyclohexene oxides with dialkyl and diaryl hydrogen phosphates as a model for the reaction for BP diol epoxide with nucleic acids. Reactions of epoxides with hydrogen phosphates have been sporadically studied before[7], but there appears to be no systematic investigation.

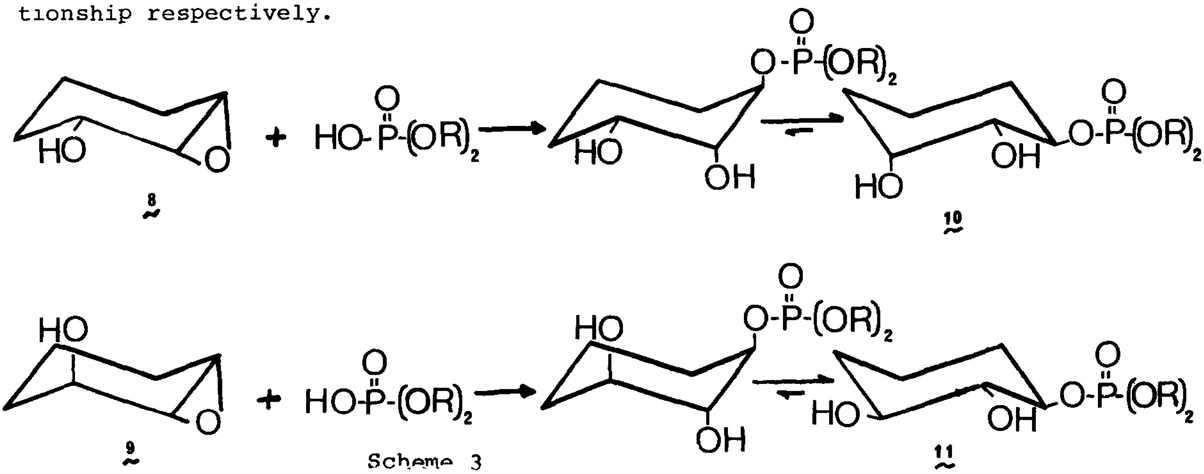
Cyclohexene oxide (4) reacts with a number of hydrogen phosphates (5) to give in good yield esters of *trans*-2'-hydroxy-cyclohexyl phosphate (6) (scheme 2). Reaction of 4 with diphenyl phosphate (5a) in benzene is a typical example[8]. The product, 6a, a crystalline solid of m.p. 89-91°, was obtained in 80% yield after work up. Its structure was confirmed by chemical ionization mass spectrometry (isobutane) which showed a M+1 peak at m/e = 349. Its ^{13}C nmr spectrum was particularly informative in the cyclohexyl carbon region: C_1' = 84.82 (J_{C-P} = 6.84 Hz) C_2' = 72.74 (J_{C-P} = 4.88 Hz), C_3' = 32.37, C_4' = 23.65, C_5' = 23.30 and C_6' = 31.27 (J_{C-P} = 2.93 Hz). The *trans*-opening of the epoxide was confirmed by an independent preparation of 6a from *trans*-1,2-cyclohexanediol with equimolar amounts of diphenyl chlorophosphate (7) and pyridine in benzene. Reaction of a mixture of *cis*- and *trans*-1,2-cyclohexanediol with 7 gave a mixture of *cis*- and *trans*- 6a.

Cyclohexene oxide also reacts with anilinium diphenyl phosphate in benzene to give 6a, albeit in somewhat diminished yield (~ 50%)



scheme 2

The reactions of phosphodiesters with *cis*- and *trans*-cyclohexenol 2,3-oxide (8,9)[10] were quite interesting (scheme 3). In all cases, the opening of the epoxides was found to be stereospecific *trans*-opening giving the products 10 and 11. The reaction was also regiospecific, with the phosphate attacking the 3' position, away from the neighbouring hydroxy group. The molecular weight of all the products was determined by CI-MS. The regiospecificity of the reaction is evident from the ^{13}C nmr spectra of the products. Of the three carbinol carbons in the cyclohexyl ring, two of them are coupled to phosphorus (Table). If the opening had occurred at the 2' position, all three carbons would have coupled with phosphorus. The stereospecific *trans* opening was deduced from the similarity in the J_{C-P} coupling constants of 10, 11 and those of 6 for the carbinol carbons. The chemical shifts of all the cyclohexyl carbons in 10 are at higher field than the corresponding carbons in 11, thus indicating the presence of an axial substituent in 10[11]. The ^1H nmr spectra of 10a and b are also in agreement with the stereochemistry assigned. The carbinol hydrogen at carbon 2' appears as doublet of doublet with H-H coupling constants of 7.6 Hz and 2.7 Hz, indicating an axial-axial and an axial-equatorial relationship respectively.



Scheme 3

At the moment, the following conclusion can be drawn. Phosphodiesters can be converted efficiently to phosphotriesters by cyclohexene oxides. The same conversion can be achieved with amine salts of these phosphates. This suggests that reaction of BP diol epoxide with nucleic acids as envisaged by scheme 1 is indeed chemically feasible. Furthermore, it has been suggested that the phosphate ester formation at the C-10 position of BP diol epoxide most likely proceeds through an S_N1 mechanism[5]. Our results suggest that under acidic conditions, the presence of a neighbouring hydroxy group is sufficient to confer regiospecificity on the attack of the phosphate on the epoxide in an S_N2 mechanism.

Study on the reactions of epoxides with nucleotides will be reported in due course.

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References and Footnotes

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8. Typical procedure: The epoxide (1.6 mmol) was added dropwise to a solution of hydrogen phosphate (1.6 mmol) in 2 ml benzene at room temperature. The reaction mixture was stirred for 40 minutes. The mixture was taken up in 15 ml CH_2Cl_2, washed with saturated sodium bicarbonate solution and water and dried. After evaporation of solvent, the product was obtained as a solid or an oil. (∼ 80% yield).
9. Reaction of cyclohexene oxide with dibenzyl hydrogen phosphate was reported to give trans-opening of epoxide[7]. The stereochemistry was never vigorously proven as far as we could determine.
10. Prepared according to H.B. Henbest and B. Nicholls, J. Chem. Soc., 4608 (1957) and C.G. Chavdarian and C.H. Heathcock, Syn. Comm., 6, 277 (1976).
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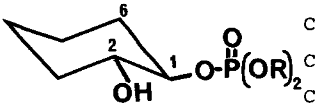
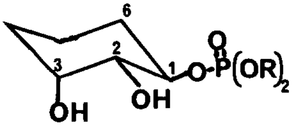
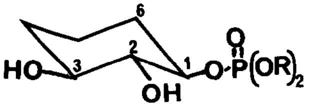
Table: ^{13}C nmr of Cyclohexyl phosphates ($CDCl_3$), δ in ppm (J_{C-P} in Hz)

		a: R = Ph	b: R = CH_2Ph	C: R = CH_2CH_3
 6	C_1	84.82 (6.84)	83.35 (6.83)	83.11 (6.10)
	C_2	72.74 (4.88)	73.00 (4.88)	74.58 (4.88)
	C_6	31.27 (2.93)	31.46 (3.40)	31.46 (3.66)
 10	C_1	81.41 (6.81)	79.73 (6.84)	79.09 (7.32)
	C_2	74.36 (3.91)	74.86 (2.93)	74.64 (3.66)
	C_3	69.91	69.79	69.80
	C_6	30.42 (3.91)	30.88 (4.88)	30.70 (4.88)
 11	C_1	82.40 (6.84)	80.96 (5.86)	
	C_2	78.74 (4.88)	78.98 (3.90)	
	C_3	72.85	73.02	
	C_6	31.28 (4.88)	31.46 (3.90)	

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