The dialyzed enzyme solution was purified by the column chromatography with diethylaminoethyl (DEAE)-cellulose (3.0×10 cm). The enzyme solution was applied onto the column which had been equilibrated with the same buffer as used in dialysis. After washing the column, alkaline phosphatase was eluted by changing the concentration of NaCl gradiently from 0 to 0.3M, and the active fraction was collected and concentrated by the membrane filter.

The enzyme solution was dialyzed against 10 mM citrate buffer (pH 5.0) containing 10 μM $ZnCl_2$ and $MgCl_2$ for 6 hr. The solution was passed a column (2.0×30 cm) of carboxymethyl (CM)-cellulose which had been equilibrated with the same buffer as used in dialysis and the effluent was collected. After concentration, the solution was dialyzed against 10 mM Tris buffer (pH 8.0) containing 10 μM of $ZnCl_2$, $MgCl_2$ and 0.1M NaCl.

The dialyzed enzyme solution was purified on a column (2.5×100 cm) of Sephadex G-200 which had been equilibrated with same buffer as used in dialysis. Above purification procedures were summarized in Table I.

Human liver alkaline phosphatase was purified about 8900-fold based on the acetone precipitate level with recovery of 10%. The resulting enzyme gave a single band of protein by disc electrophoresis using 7.5% polyacrylamide gel at pH 9.4 as shown in Fig. 1.

Enzymological and immunological properties will be reported successively.

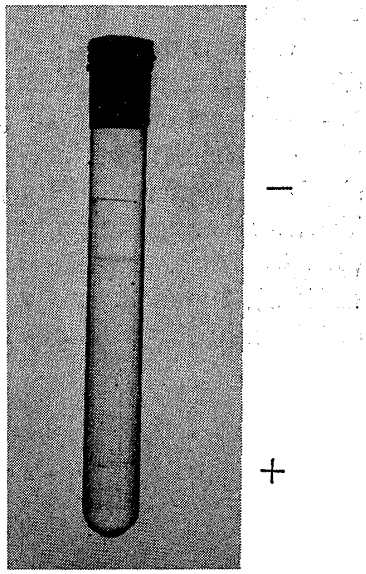

Fig. 1. Electrophoretic Pattern of Purified Alkaline Phosphatase from Human Liver

Electrophoresis was carried out at pH 9.4 under a constant current of 4 mA/tube for 70 min.

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Alkylation of Phenols by Phenanthrene 9,10-Oxide[1)]

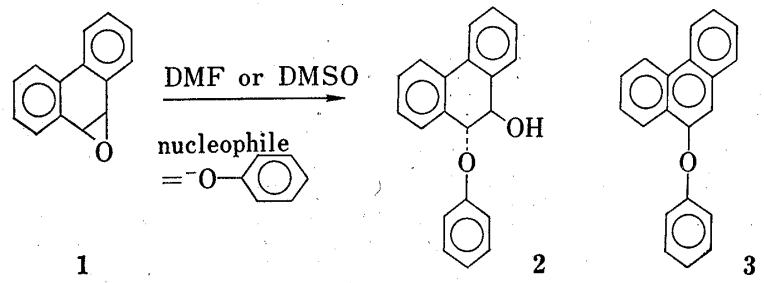
The reactions of phenanthrene, 9,10-oxide (1) and phenols or phenoxides were investigated. In dipolar aprotic solvents, 1 alkylates only the oxygen atom of the ambident phenoxide. In protic solvents, C-alkylation of phenol is observed.

The recognition that arene oxides may play a significant role in carcinogenesis by polynuclear hydrocarbons is growing. The reaction between polycyclic arene oxides and biomolecules such as nucleic acid bases or protein residues, is thought to be responsible for the

1) Paper 4 in a series on the Chemistry of Carcinogenic Functional Groups. See the previous paper, K. Shudo and T. Okamoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 2809 (1973).

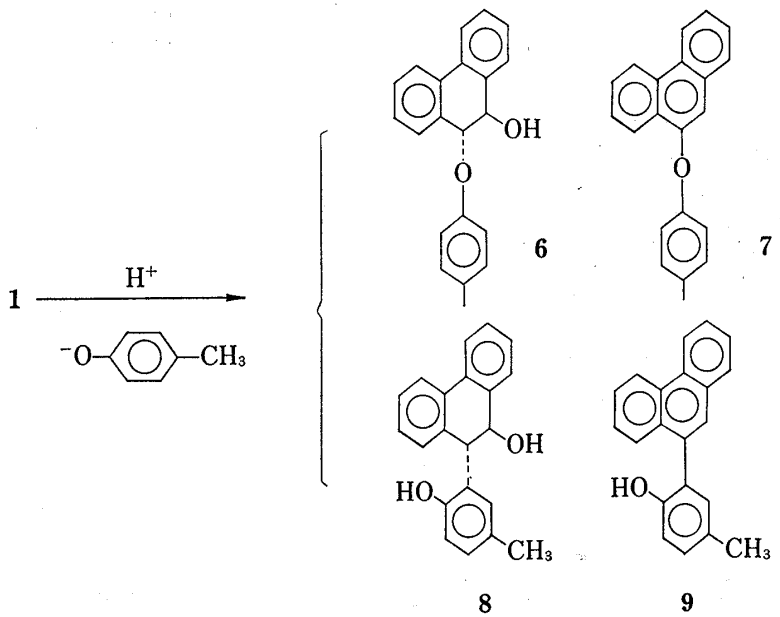
emergence of carcinogenicity.[2] On the other hand reactivity of polycyclic arene oxides towards nucleophiles has not been studied in organic chemistry.[3] We wish to present experimental evidences indicating electrophilic nature of an arene oxide towards phenoxides under a wide range of reaction conditions. The selectivity of C- and O-alkylation will help to discuss the mode of interaction between arene oxides and biomolecules.

When a mixture of phenanthrene-9,10-oxide (**1**) and sodium phenoxide was heated in dimethyl formamide (DMF) at 100° in nitrogen atmosphere, 9,10-dihydro-*trans*-9-hydroxy-10-phenoxyphenanthrene (**2**, 19%), 9-phenoxyphenanthrene (**3**, 68%),[4] and a trace of 9-phenanthrol (**4**) and phenanthrene 9,10-quinone were identified. In the presence of air, a significant amount of 9-hydroxy-10-phenoxyphenanthrene was formed instead of **2** and **3**. The *trans* configuration for **2** was suggested from nuclear magnetic resonance (NMR) spectrum ($J_{9,10}$=10 Hz). **2** was transformed into **3** under the reaction conditions. In dimethyl sulfoxide (DMSO), **3** was the major product and **2** could not be isolated. Very similar results were obtained in the reactions between **1** and sodium salts of *p*-cresol, α- and β-naphthols.[5]



1 2 3

Fig. 1



6 7

8 9

Fig. 2

2) P. Brookes, W.M. Baird, and A. Dipple, "Chemical Carcinogenesis," Part A. ed. by P.O. Ts'o and J.A. DiPaolo, Dekker, N.Y, 1974, p. 149; P.L. Grover, and P. Sims, *Biochem. Pharmacol.*, **19**, 2251 (1970); T. Kuroki, E. Huberman, H. Marguardt, J.H. Selkirk, C. Heidelberger, P.L. Grover, and P. Sims, *Chem.-Biol. Interactions* **4**, 389 (1971/1972).

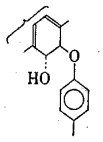
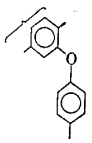
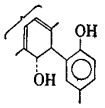
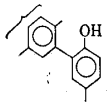
3) For monocyclic oxides, see, D.M. Jerina, "Chemical Carcinogenesis," Part A. 1974, p. 294; D.M. Jerina, *Heterocycles*, **1**, 267 (1973).

4) G. Wittig, W. Uhlenbrood, and P. Weinhold. *Ber.*, **95**, 1698 (1962).

5) Pottasium cyanide and sodium bromide react with **1** in DMF, which yield 9-cyanophenanthrene and 9-phenanthrol, respectively. Without any nucleophile, the decomposition of the epoxide to 9-phenanthrol is slow.

Since *p*-cresol is a better model for the tyrosine residue, we studied the alkylation of *p*-cresol or cresolate with **1** in detail, with the purpose of finding whether C-alkylation occurs or not. Under the S_N2 condition (in DMF or DMSO), the alkylation of sodium cresolate occurred exclusively at the oxygen atom, but not at the carbon atoms of the ring.[6] Thus, **6** and **7** were identified. The addition of a protic solvents did really cause the C-alkylation, though the yields of C-alkylation products (**8** and **9**) were low. In acetone–water or dioxane–water, the yield of C-alkylation products increased up to 2%. C-Alkylation was observed in the reaction catalysed by trifluoroacetic acid or boron trifluoride etherate at room temperature.[7] The structure of **9** was determined by an independent synthesis[8] as its methyl ether. The compound (**8**) was transformed into **9** by dil. hydrochloric acid. The stereochemistry of the 9,10-position was deduced to be *trans* from NMR.

TABLE I. Reaction of Phenanthrene oxide and *p*-Cresol

Conditions[a,6]	 **6**	 **7**	 **8**	 **9**	**8+9**
DMSO–CrONa	—%	55.7%	—%	<0.01%	<0.01%
DMSO–H_2O(10%)–CrONa	0.1	54.7	0.01	0.18	0.2
DMSO–H_2O(50%)–CrONa	73.2	0.7	0.86	0.08	0.9
DMSO–EtOH(50%)–CrONa	1.7	48.4[b]	0.01	0.06	0.1
DMSO–CrOH(50%)–CrONa	54.2	0.7	0.42	0.15	0.6
Acetone–H_2O(50%)–CrONa[c]	86.4	1.2	1.77	0.31	2.1
Dioxane–H_2O(50%)–CrONa	89.5	0.7	1.91	0.40	2.3
CF_3CO_2H–CrOH[d]	—	2.5	—	2.44	2.4
$BF_3 \cdot Et_2O$–CrOH[d,6]	—	54.8	—	5.20	5.2

a) in a sealed tube at 100°, in N_2. 5 hr
b) a mixture with 9-ethoxyphenanthrene
c) at reflux for 5 hr
d) at room temperature

The observed C-alkylation in protic media may be a reflection of the A2 mechanism,[9,10] which involves a protonated epoxide, or a more polarised transition state than in S_N2 mechanism. The solvation of the oxygen atom of the nucleophile in addition to the possible change of the reaction mechanism, may have an important role in the determination of alkylation sites.[11] The present study may suggest that the C-alkylation by polycyclic arene oxides as well as O-alkylation should be considered in the biological reactions.[12]

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6) The ylelds of C-alkylation products were determined by GLC after isolation by TLC. The yields of O-alkylation products were the isolated ones by TLC.
7) Since the yield of the reaction catalyzed by BF_3 seems to be depend on the experimental conditions used, we need further experiments.
8) Ullman condensation between 9-bromophenanthrene and 3-iodo-4-methoxytoluene in the presence of CuO and CuZn in DMF.
9) J.B. Buchanan and H.Z. Sabel, "Selective Organic Transformation," Vol. 2, ed. by B.S. Thyagarajan, Wiley-Interscience, New York, 1972, p. 1.
10) R.E. Parker and N.S. Isaacks, *Chem. Rev.*, **59**, 737 (1959).
11) H.O. House, "Modern Synthetic Reactions," 2nd. ed., Benjamin, Menlo Park, Calif., 1972, p. 492.
12) C-Alkylation by some carcinogenic hydroxylamine derivatives has been shown, J.A. Miller, *Cancer Research*, **30**, 559 (1970).