[Chem. Pharm. Bull. 27(10)2547—2549(1979)]

UDC 547.838.1.04.09 : 615.277.4.011.5.074

Identification of an Ultimate Mutagen of 10-Azabenzo[*a*]pyrene: Microsomal Oxidation of 10-Azabenzo[*a*]pyrene to 10-Azabenzo[*a*]pyrene-4,5-oxide

10-Azabenzo[*a*]pyrene (10-ABP), one of most active mutagens, was incubated with microsomal protein. 10-ABP-4,5-oxide was found by high performance liquid chromatography in the presence of epoxide hydratase inhibitor, 3,3,3-trichloropropyrene-1,2-oxide (TCPO). In the absence of TCPO, formation of 10-ABP-4,5-oxide was almost negligible and *trans*-4,5-dihydroxy-4,5-dihydro-10-ABP was found as a major metabolite.

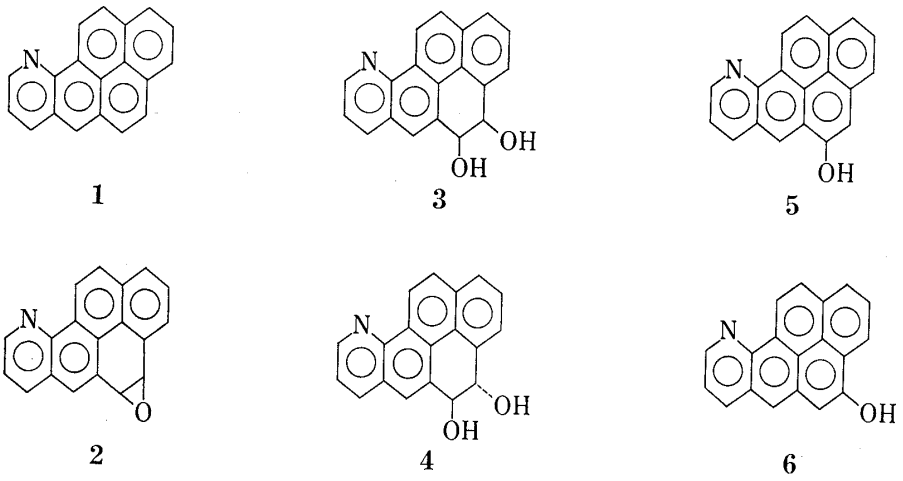
Keywords——arene oxide; 10-azabenzo[*a*]pyrene; 10-azabenzo[*a*]pyrene-4,5-oxide; mutagen; carcinogen; microsomal oxidation; metabolite; high performance liquid chromatography

Potent carcinogenic polycyclic aromatic hydrocarbons (PAH) have been intensively studied. A large effort has been made to the study of benzo[*a*]pyrene (BP).[1] However a current trend of chemical carcinogenesis studies of PAH is turning to other PAHs such as 7,12-dimethylbenz[*a*]anthracene,[2] 3-methylcholanthrene[3] and 5-methylchrysene.[4] On the other hand, chemical and biological studies of aza-analogs of PAHs are quite poor, though they are carcinogens and environmental substances.[5] It was recently demonstrated that the pyrolysate of L-lysine contains an aza-PAH, 3,4-cyclopentenopyrido[3,2-*a*]carbazole, as one of principal mutagenic compounds.[6] We also reported the syntheses, chemical reactivities and mutagenic activities of several aza-PAHs and their K-region oxides.[7,8] Among them, 10-azabenzo[*a*]pyrene (10-ABP, **1**) is one of the most active mutagens, whose activity is as potent as BP. Carcinogenecity of 10-ABP has been reported.[9] The present paper describes the microsomal oxidation of 10-ABP to its 4,5-oxide.

10-Azabenzo[*a*]pyrene-4,5-oxide (**2**) was already prepared and proved to be a very potent direct mutagen to *Salmonella typhimurium* TA 98 and 100.[8] *cis*-4,5-Dihydroxy-4,5-dihydro-10-ABP (**3**) was prepared by OsO_4 oxidation of **1**. *trans*-4,5-Dihydroxy-4,5-dihydro-10-ABP (**4**) was prepared by a careful alkaline hydrolysis of the oxide (**2**). Trifluoroacetic acid treatment of **2** gave 5-hydroxy-10-ABP (**5**), whose structure was deduced from the direction of the acid-catalyzed ring opening of benzo[*f*]quinoline-5,6-oxide.[7] A mixture of monoacetates of **3** was treated with methanesulfonyl chloride to give a mixture of phenol acetates. Fractional recrystallization of the mixture and hydrolysis gave the other phenol, 4-hydroxy-10-ABP (**6**).

1) T.J. Slaga, W.M. Bracken, A. Viaje, D.L. Berry, S.M. Fisher, D.R. Miller, W. Levin, A.H. Conney, H. Yagi, and D.M. Jerina, "Carcinogenesis, A Comprehensive Survey," Vol. 3, (Polynuclear Aromatic Hydrocarbons), Reven Press, New York, 1978, pp. 371—382; S. K. Yang, P. P. Roller, and H. V. Gelboin, "Carcinoglnesis, A Comprehensive Survey," Vol. 3, (Polynuclear Aromatic Hydrocarbons), Reven Press, New York, 1978, pp. 285—301.
2) S.K. Yang and M.W. Chou, *J. Am. Chem. Soc.*, **101**, 239 (1979); K.B. Sukumaran and R.G. Harvey, *J. Am. Chem. Soc.*, **101**, 1353 (1979).
3) T.A. Stoning and R.J. Gerardot, *Life Sciences*, **20**, 113 (1977).
4) S. Brynes, G.J. Burckart and M. Mokotoff, *J. Med. Chem.*, **21**, 45 (1978).
5) A. Dipple, "Chemical Carcinogens," ed. by C.E. Searle, American Chemical Society, New York, 1976, Chapter 5; E. Sawacki, J.E. Miller, and M.J. Morgan, *Int. J. Air Wat. Poll.*, **9**, 291 (1965).
6) K. Wakabayashi, K. Tsuji, T. Kosuge, K. Yamaguchi, K. Shudo, Y. Iitaka, T. Okamoto, T. Yahagi, M. Nagao, and T. Sugimura, *Proc. Japan Acad.*, **56**, 569 (1978).
7) Y. Kitahara, K. Shudo, and T. Okamoto, *Heterocycles*, **8**, 363 (1977).
8) Y. Kitahara, H. Okuda, K. Shudo, T. Okamoto, M. Nagao, Y. Seino, and T. Sugimura, *Chem. Pharm. Bull.* (Tokyo), **26**, 1950 (1978).
9) A. Lacassagne, N.P. Buu-Hoi, F. Zajdela, and P. Mabille, *C.R. Acad. Sci. Paris*, **258**, 3378 (1964).

NII-Electronic Library Service



1 3 5

2 4 6

Chart 1

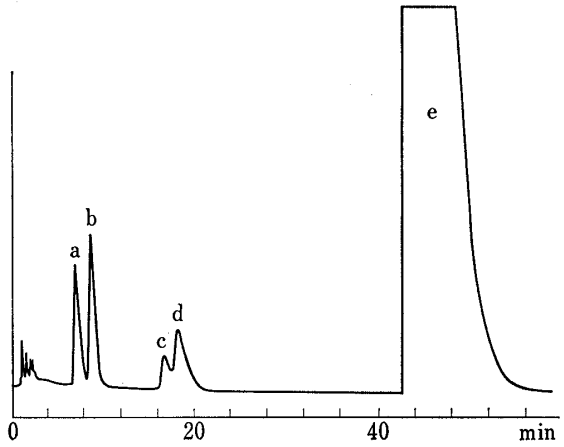


Fig. 1. High Performance Liquid Chromatogram of 10-ABP Metabolites in the Presence of TCPO

Column; LiChrosorb RP-18, 4.6ϕ × 150 mm.
Solvent; MeOH-water-NEt_3 (75: 25: 0.01).
Flow rate; 1.0 ml/min.
Peaks a—d, see text. Peak e is 10-ABP.

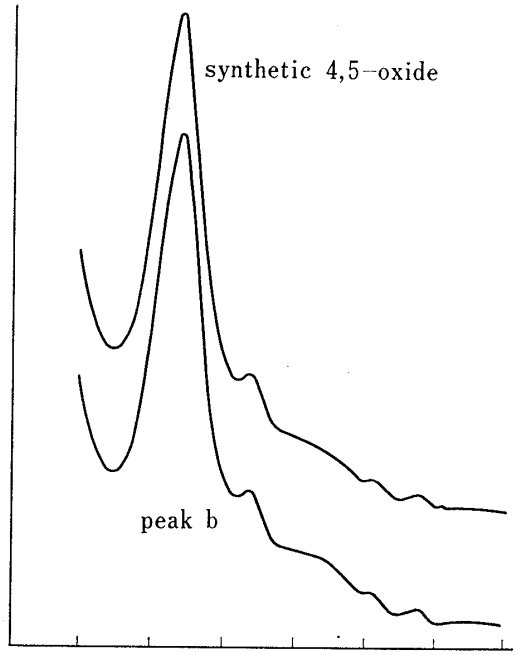


Fig. 2. Ultraviolet Spectra of Peak b and Synthetic 4,5-Oxide

The microsomal oxidation products of 10-ABP were obtained by *in vitro* incubation of a suspension of 10-ABP with liver microsomes from polychlorobiphenyl-treated Wistar rats at 37° for 10 min.[10] The shorter incubation time simplified the composition of products. For the purpose of epoxide identification, the incubation mixture included 2 mM 3,3,3-trichloropropyrene-1,2-oxide (TCPO), an epoxide hydratase inhibitor. The substrate and metabolites were extracted with dichloromethane and analyzed by high performance liquid chromatography (HPLC). An example of chromatographic patterns in the presence of TCPO is illustrated in Fig. 1. Retention times with use of different columns[11] and ultraviolet

10) The incubation mixture contained, in a total volume 400 ml, 1.2 mmol of $MgCl_2$, 0.04 mmol of 10-ABP dissolved in 20 ml ethanol, 0.02 mol of Tris-HCl (pH 7.5), 100 mg of microsomal protein and 0.144 mmol of NADPH.

11) LiChrosorb RP-18, solvent; MeOH–water–NEt_3 (75: 25: 0.01). LiChrosorb Rp–NH_2, solvent; hexane–chloroform (9: 1).

NII-Electronic Library Service

spectrum of the peak b (Fig. 2) were compared with those of the synthetic 4,5-oxide (**2**). Weak acid-treatment in dichloromethane of the isolated peak b and the authentic 4,5-oxide gave a similar mixture of **5** (about 85%), **3** (about 10%) and a trace of **4**. HPLC analysis of the metabolite mixture did not indicate the presence of **5** and **6**, though peak a was probably a phenolic compound.[12] Peaks c and d were found regardless of the presence of TCPO. These peaks appear rather nonpolar since their HPLC retention times were found between **1** and **2**, while they are resistant to the treatment by 1% trifluoroacetic acid which is acidic enough to isomerize an epoxide. These results suggest that peaks c and d are not epoxide.

In the absence of TCPO the formation of the 4,5-oxide was almost negligible, but instead, *trans*-dihydrodiol (**4**) was found as a major metabolite, whose identification was performed by HPLC retention time and ultraviolet spectrum.

The present result provides a significant background for a further study on the 4,5-oxide as one of active mutagenic metabolites of 10-ABP. Since a current concept, the so-called bay region theory,[13] cannot be applied to the present compound, understanding of molecular mechanism about the interaction of the activated form(s) with DNA is expected.

Faculty of Pharmaceutical Sciences
University of Tokyo
Hongo, Bunkyo-ku, Tokyo, 113, Japan

Haruhiro Okuda
Yoshiyasu Kitahara
Koichi Shudo
Toshihiko Okamoto

Received July 16, 1979

12) The retention times of the peak a, as well as phenols **5** and **6**, were very sensitive to the amount of NEt_3.
13) D.M. Jerina, and J.W. Daly, "Drug Metabolism," ed. by D.V. Parke, and R.L. Smith, Taylar and Francis, London, 1977, pp. 13—33.

NII-Electronic Library Service