Available online at www.sciencedirect.com

SCIENCE DIRECT®

Talanta 69 (2006) 1260–1264

Talanta

www.elsevier.com/locate/talanta

Effect of octabromination of a tetrakis(4-carboxyphenyl)porphine derivative bound to silica gels on HPLC retention behaviors of polyaromatic hydrocarbons

Youji Kitamura [a], Kenji Kawata [b], Kurumi Tanaka [b], Yuko Furuyashiki [b], Masaki Mifune [a,*], Ikuko Tsukamoto [c], Madoka Saito [b,c], Jun Haginaka [d], Yutaka Saito [a]

[a] *Department of Pharmaceutical Sciences, Graduate School of Medicine and Dentistry and Pharmaceutical Sciences, Okayama University, Tsushima-Naka, Okayama 700-8530, Japan*

[b] *Department of Pharmaceutical Chemistry, Graduate School of Natural Science and Technology, Okayama University, Tsushima-Naka, Okayama 700-8530, Japan*

[c] *Faculty of Medicine, Kagawa University, Ikenobe, Miki-Cho, Kagawa 761-0793, Japan*

[d] *Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Koshien, Nishinomiya 663-8179, Japan*

Received 15 November 2005; received in revised form 28 December 2005; accepted 28 December 2005
Available online 10 February 2006

Abstract

The effect of bromination of Cu-porphyrin-derivative-immobilized silica gels (Cu-$TCPP_D$) was examined by comparing the retention behaviors of polyaromatic hydrocarbons (PAHs) on Cu-$TCPP_D$ and Cu-octabromotetrakis(4-carboxyphenyl)porphine-derivative-immobilized silica gels (Cu-$OBTCPP_D$) columns. It was revealed that bromination affects strongly the π–π electron interactions caused from hydration energy in a polar eluent (80% methanol) possibly as a result of a destruction of planar structure of porphine-ring by bromination. It was also revealed that bromination enhances π–d interactions as well as the π–π electron interactions in a broad sense (e.g., dispersion forces) in a non-polar eluent (*n*-hexane). However, the bromination did not exert much influence on electrostatic interactions caused from polarization of mono-halogenated benzenes.

Keywords: HPLC stationary phase; π–d Interaction; π–π Interaction; Bromination of porphyrin; Dispersion force; Polyaromatic hydrocarbons

1. Introduction

In the separation and/or analysis of mutagens and environmental pollutants, such as polyaromatic hydrocarbons (PAHs) characterized by the presence of π electrons, HPLC stationary phases which achieve separation through π electron interactions have proved to be useful [1–5]. Typical columns of this type are a PYE column containing a stationary phase having 2-(1-pyrenyl)-ethyldimethylsilyl group and analogues thereof [6]. Besides, columns on which macrocyclic compounds with a wide spread of π electron-cloud such as Cu-phthalocyanine derivatives [7,8] or In-protoporphyrin [9,10] have been developed and reported to be useful for the separation of PAHs and the like. The Cu-phthalocyanine-derivative-immobilized silica gels columns are known to exert different interactions depending on an eluent due to the presence of macrocyclic aromatic ring. That is, in a polar eluent, it exerts strong π–π electron interactions, and, in a non-polar eluent, interactions involving π electron wherein π electrons of PAHs coordinate to Cu atom, or the like [8,11]. These stationary phases are characterized by a large planar π electron-cloud structure. On the other hand, it has been known that octabromination of porphine-rings destroys the planar structure [12,13]. In the present research, we attempted to evaluate the effect of bromination of porphine-ring on the retention behavior of PAHs, etc., in comparative experiments using Cu-tetrakis(4-carboxyphenyl)porphine- and octabromo-analogue-immobilized silica gels ("Cu-$TCPP_D$" and "Cu-$OBTCPP_D$") columns.

* Corresponding author. Tel.: +81 86 251 7952; fax: +81 86 251 7953.
E-mail address: mifune@pharm.okayama-u.ac.jp (M. Mifune).

0039-9140/$ – see front matter
doi:10.1016/j.talanta.2005.12.049

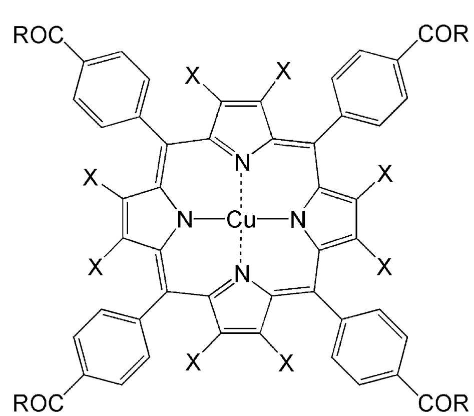
2. Experimental

2.1. Chemicals and reagents

Tetrakis(4-carboxyphenyl)porphine (H_2-TCPP) was obtained from Tokyo Kasei Co. Ltd., Japan. As a support, Develosil NH_2-5, which is spherical aminopropyl-silica gel (particle size, 5 μm; specific surface area, 250 m^2/g; average pore size, 12 nm) was purchased from Nomura Kagaku, Japan. As eluents, purified water by Labo IonPure-12 (Millipore, USA), methanol and *n*-hexane of an HPLC-grade (Nacalai Tesque, Kyoto, Japan) were mainly used. As a solvent for packing Cu-$TCPP_D$ and Cu-$OBTCPP_D$, slurry solvent A Conc. (Chemco Scientific, Japan) was used. The commercially available 2-(1-pyrenyl)ethyldimetylsilyl silica gels (PYE) column was purchased from Nacalai Tesque. Other reagents were of analytical or reagent grade.

2.2. Preparation of Cu-$TCPP_D$ and -$OBTCPP_D$ columns

Cu-TCPP and Cu-OBTCPP (see Fig. 1) was synthesized according to the method described in literature [14–16]. Acid chlorides of Cu-TCPP and Cu-OBTCPP (Cu-TCPPCl and Cu-OBTCPPCl, see Fig. 1) was synthesized from dry Cu-TCPP and Cu-OBTCPP as described by Iwado et al. [17]. To prepare the Cu-porphyrin-immobilized silica gels, Cu-TCPPCl or Cu-OBTCPPCl (ca. 4 mg) was dissolved in dry dioxane (10 ml). After adding Develosil NH_2-5 (ca. 1.5 g), the mixture was refluxed for 2 h and allowed to cool. Cu-$OBTCPP_D$ or Cu-$OBTCPP_D$ (15 μmol Cu-porphyrin/g Dev) was filtered off and washed with methanol (100 ml), dried thoroughly in vacuo over P_4O_{10}. The Cu-$TCPP_D$ and Cu-$OBTCPP_D$ columns were prepared by packing the resulting silica gels into a stainless steel column (4.6 mm i.d. × 150 mm) by a conventional slurry packing method. The amount (15 μmol/g) of Cu-TCPPCl and Cu-OBTCPPCl immobilized was estimated from the absorption spectrum of the initial Cu-TCPPCl and Cu-OBTCPPCl solution in dry dioxane.



X=H, R=OH : Cu-Tetrakis(4-carboxyphenyl)porphine (Cu-TCPP)

X=Br, R=OH : Cu-Octabromotetrakis(4-carboxyphenyl)porphine (Cu-OBTCPP)

X=H, R=Cl : Acid chloride of Cu-TCPP (Cu-TCPPCl)

X=Br, R=Cl : Acid chloride of Cu-OBTCPP (Cu-OBTCPPCl)

Fig. 1. Structures of Cu-porphyrins.

2.3. Apparatus

The HPLC system consisted of a Shimadzu LC-10AT pump, a Shimadzu SPD-10A detector, a Shimadzu Chromatopac C-R6A recorder (Shimadzu Co., Japan) and a Rheodyne model 7161 sample injector (Rheodyne, USA). Typical HPLC conditions are as follows: column temperature, ambient; detection, 264 nm in principle; an eluent, 50–90% methanol or 100% *n*-hexane at a flow rate of 0.5 ml/min.

2.4. Samples

Sample compounds include one to four-membered PAHs shown in Fig. 2 (Kanto Kagaku, Tokyo Kasei, Nacalai Tesque and Wako Junyaku, Japan), fluorobenzene, chlorobenzene, iodobenzene (Nacalai Tesque), bromobenzene, benzonitrile (Ishidzu Pharm. Co. Ltd., Japan) and nitrobenzene (Katayama Chem. Ind., Japan). Sample solutions were 250 μg/ml benzene and its derivatives, and 5–20 μg/ml PAHs solutions in 80% methanol or 100% *n*-hexane.

3. Results and discussion

In both of the Cu-$TCPP_D$ and Cu-$OBTCPP_D$, Cu-porphyrins are supposed to be bound in parallel with the surface of the silica gel through the four amide bonds. In such a situation, a sample having π electrons is expected to show interactions involving π electrons with Cu-porphyrins having a wide spread of π electron. Therefore, bromination that destroys the planarity of Cu-TCPP may affect the interactions with a sample involving π electrons. From this viewpoint, comparative experiments were conducted to elucidate the effect of bromination on the retention behaviors of PAHs. Specifically, the retention behaviors of PAHs, etc., on the Cu-$OBTCPP_D$ and Cu-$TCPP_D$ columns were compared and the change of interactions was elucidated.

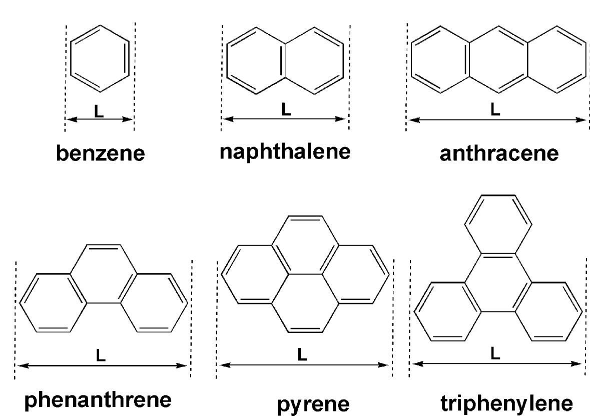


Fig. 2. Structures of polyaromatic hydrocarbons (PAHs). L: the longest molecular length, see Section 3.1.