Journal of Chromatography A, 1281 (2013) 26–31

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Probing the chiral separation mechanism and the absolute configuration of malathion, malaoxon and isomalathion enantiomers by chiral high performance liquid chromatography coupled with chiral detector–binding energy computations

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ARTICLE INFO

Article history:
Received 29 October 2012
Received in revised form
25 December 2012
Accepted 3 January 2013
Available online 18 January 2013

Keywords:
Chiral separation
Cotton effect
Optical dispersion
Absolute configuration
Binding energy computation

ABSTRACT

Chiral separation mechanism determination and absolute configuration assignment are fundamental to the development of chiral stationary phases (CSPs) and the evaluation of both the enantioselective bioactivity and fate of chiral compounds. This work investigated the process of chiral separation and the assignment of the absolute configurations of malathion, malaoxon, and isomalathion using chiral high performance liquid chromatography (HPLC) coupled with chiral detector–binding energy computations. Hydrogen bonding was found to be a very important factor in the chiral separation of isomalathion on Chiralpak AD, although it did not exhibit a significant effect on the chiral separation of malathion and malaoxon on Chiralcel OJ. Based on the sign of a chiral detector, the relationships between the cotton effect, optical dispersion and absolute configuration were established for individual enantiomers of malathion, malaoxon, and isomalathion. The elution orders of the enantiomers of malathion and malaoxon on Chiralcel OJ and the stereoisomers of isomalathion on Chiralpak AD predicted by binding energy computations were found to coincide precisely with those observed in the chiral separation experiments. The result suggests that binding energy computations can be used to assign the absolute configuration of the enantiomers of chiral compounds eluted on CSPs.

1. Introduction

Malathion (*S*-[1,2-*bis*(ethoxycarbonyl)ethyl] *O*,*O*-dimethyl phosphorodithioate) is an organophosphorous insecticide (OP) compound that has been widely used for controlling insects on fruits and vegetables. It also has been used to control miscellaneous household insects, animal parasites, and lice on the human head and body [1,2]. Malathion can be bioactivated to malaoxon (*O*-[1,2-*bis*(ethoxycarbonyl)ethyl] *O*,*O*-dimethyl phosphorodithioate) via oxidation desulfuration by insect metabolism and then is transformed to isomalathion (*S*-[1,2-*bis*(ethoxycarbonyl)ethyl] *O*,*S*-dimethyl phosphorodithiolate) by thermal or photochemical isomerization. Isomalathion has been identified in certain commercial formulations and is suspected to be a prime agent in the death of 5 workers and the sickening of another 2800 in Pakistan during a 1976 malaria-eradication program [3]. Malathion and malaoxon contain an asymmetric carbon atom, which leads to the formation of two enantiomers, respectively. The isomerization of malathion to isomalathion not only maintains the asymmetric carbon atom but also forms a new asymmetric phosphorus atom, yielding four possible stereoisomers (Fig. 1) [4]. At present, malathion is still marketed and applied in its racemic form despite the fact that the (*R*)-enantiomer shows a higher biological activity than the (*S*)-isomer [5–7].

Enantioselective separation is very important in the assessment of the activity and environmental fate of individual enantiomers of chiral pesticides. To date, the enantiomers of malathion have been successfully resolved by high-performance liquid chromatography (HPLC) using polysaccharide chiral stationary phases (CSPs) [6,8–10]. Complete separation for malaoxon enantiomers has been achieved on the Chiralcel OJ column and for isomalathion enantiomers, on the Chiralpak AD column. However, the possible mechanism of chiral separation was not thoroughly discussed in these previous studies. Although it is very important to compare the enantioselectivity in separation and the biological effect indicated by different chiral terms as well as to characterize the absolute

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http://dx.doi.org/10.1016/j.chroma.2013.01.016

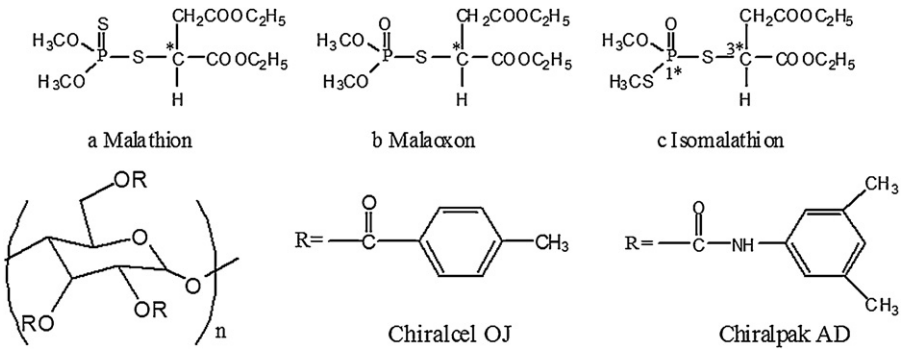

Fig. 1. Molecular structures of compounds and CSPs. * chiral center.

configuration of trace enantiomers prepared by HPLC with chiral detectors, nonetheless the relationships among the cotton effect (CE), the optical rotatory dispersion and the absolute configuration of an individual enantiomer have not been established. It is noted that the absolute configuration of resolved enantiomers cannot be directly assigned by the present chiral detectors. Thus, the assignment of the absolute configuration of resolved enantiomers only depends on other techniques. With the development of computer-based techniques, computational methods have been successfully used to provide insight into the mechanism of chiral recognition between the enantiomer and the CSPs [11–13]. The interaction model can provide information concerning the site and the main factors of influence in chiral recognition on the molecular level. The elution order of the enantiomers is determined by the difference in the interaction energy between the enantiomer and CSPs. With increased interactions between the enantiomer and CSP, as indicated by the absolute binding energy, the times of enantiomer elution from the CSP also increase. Thus, the interaction energy can be used to predict the elution order of the enantiomers on the CSP and to confirm the absolute configuration of the peaks.

To elucidate the process of chiral separation and to establish the relationships among the cotton effect, the optical rotatory dispersion and the absolute configuration of individual enantiomers of malathion and its metabolites, the present study was undertaken. The goal was to separate enantiomers of malaoxon and isomalathion using HPLC with variations in the CSPs, the mobile phase and the temperature and to calculate the interaction energy between each enantiomer and the corresponding CSP.

2. Experimental

2.1. Chemicals

An analytical standard of racemic malathion (purity > 95.0%) was a gift from Sinochem Ningbo Chemicals Co., Ltd. (Ningbo, China). Racemic malaoxon and isomalathion (purities > 94.0%) were purchased from Sigma Chemical Co., Ltd. (Shanghai, China). All other chemicals and solvents were of HPLC grade.

2.2. Chromatography conditions and resolution of the enantiomers

Chiral separation and chiroptical detection were performed on a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan) equipped with a CO-2060 column temperature control compartment, a variable wavelength CD-2095 circular dichroism detector (CD), and an OR-2090 PLUS optical rotatory dispersion detector (ORD) [14]. Two commercial HPLC columns, Chiralcel OJ ([cellulose tris-(4-methyl benzoate)], Daicel Chemical Industries, Tokyo, Japan) and Chiralpak AD ([amylose tris-(3,5-dimethylphenyl-carbamate)], Daicel Chemical Industries, Tokyo, Japan), were tested for their ability to resolve the enantiomers used in this study (Fig. 1). The columns were 250 mm × 4.6 mm (i.d.) in dimension with different enantioselective phases coated onto 5 μm silica gel beads. For all resolution experiments, the injected volume and the detection wavelength of the CD were fixed at 20 μL and 220 nm, respectively. The light source for ORD was a 150 W Hg–Xe lamp, and the tapered cell path was 25 mm with a volume of 44 μL. The rotation sign or cotton effect ("+" or "−") was indicated by a positive or negative peak on the chromatogram. The resolved enantiomers were also manually collected at the HPLC outlet and then were injected into an Agilent 6890 GC equipped with an Agilent 5975 mass selective detector for compound identification (Agilent Inc., Wilmington, DE, USA). A HP-5 MS fused silica capillary column (30 m × 0.25 mm ID, a film thickness of 0.25 μm, crosslinked 5% diphenyl and 95% dimethylpolysiloxane, Agilent Inc., Wilmington, DE, USA) was used with helium as the carrier gas (1.0 ml min^{-1}). The injection port temperature was 230 °C, the interface temperature was 280 °C, and the oven temperature was 210 °C. Electron impact (EI) ionization was performed at 70 eV. The MS scan range was set from 25 to 400.

2.3. Computational methods [15]

2.3.1. Energy-minimized structures of the isomers and chiral stationary phases (CSP)

The structures of the enantiomers of malathion, malaoxon and isomalathion were built in standard geometry and then minimized by means of molecular mechanics PCFF force field. A chiralcel OJ structure containing 9 monomers was constructed, and then a structure of chiralpak AD containing 12 monomers was downloaded from the previous study [16]. Each structure was terminated with a methyl group. All chemicals were modeled in their neutral form. The initial structures were simulated with molecular dynamics (MD) at 500 K, and 10 different conformations from 10 trajectories were sampled. Next, each conformation was minimized, and the conformation of the lowest energy was used for the subsequent studies. The simulation was carried out under NVT ensemble with an Andersen thermostat. The minimization process selected the conjugate gradient method and the Polak-Ribiere algorithm. The convergence was set to 0.1 kcal mol^{-1} $Å^{-1}$. All calculations were performed with the Discover module in MS-modeling software. The PCFF force field was used because it is applicable to the calculation of organic compounds and polysaccharides [17].

2.3.2. Generation of the complex binding energy

The structure of a given enantiomer was inserted into the middle pocket of the corresponding polymer chain, i.e., malathion and malaoxon corresponded to Chiralcel OJ, and isomalathion corresponded to Chiralpak AD. Next, an initial equilibration was performed with an MD run for 100 ps at 500 K to allow the