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Reversal of elution order during direct enantiomeric separation of pyriproxyfen on a cellulose-based chiral stationary phase

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ABSTRACT

The enantiomeric separation of pyriproxyfen (Sumilarv), a new insect growth regulator, was investigated on several commercially available chiral stationary phases consisting of cellulose esters coated on silica. The mobile phases were composed of *n*-hexane and alcohols. Resolution was achieved only by using a cellulose tris-(4-methylbenzoate)-coated silica gel. The enantiomeric elution order changed according to the steric bulk of the alcohols. The possible mechanisms of the reversal of elution order are discussed.

INTRODUCTION

Pyriproxyfen (Sumilarv, Fig. 1), newly synthesized in our company, has a high juvenile hormone mimic activity, and its high efficacy as a control agent for flies, mosquitoes, and cockroaches has recently been reported [1–5]. Pyriproxyfen has a chiral centre, and the (*S*)-(−)-enantiomer is the more active [6]. A detailed investigation of both its biological activity and metabolic fate could require a knowledge of the individual behaviour of each enantiomer under a variety of biological environment.

As a first step towards establishing this, a method should be developed that would allow the direct chromatographic separation of the optical isomers, preferably without derivatization. We report here the direct chromatographic resolution of the enantiomers of pyriproxyfen on a cellulose tris-(4-methylbenzoate)-coated silica gel. During this investigation we found that the elution order of the enantiomers changed according to the steric bulk of the alcohol in the mobile phase. This is the first example to be reported of a reversal of elution order, on a modified cellulose column, associated with changes in mobile phase modifiers, although only a few studies have been performed on the inversion of the enantiomeric elution order on chiral stationary phases [7–10]. The possible mechanisms of this reversal are discussed.

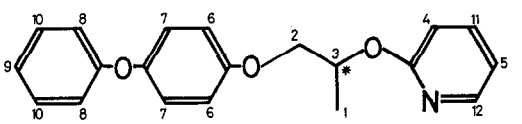

Fig. 1. Molecular structure of pyriproxyfen (Sumilarv).

EXPERIMENTAL

Apparatus

The chromatography was performed with a Hitachi (Tokyo, Japan) L-6000 pump and a Hitachi L-4000 variable-wavelength spectromeric detector. The column was stainless steel (25 cm × 4.6 mm

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I.D.), packed with cellulose tris-(4-methylbenzoate) absorbed on microporous silica (Chiralcel OJ column; Daicel, Tokyo, Japan).

Chemicals

The (*R*)-(+)- and (*S*)-(−)-enantiomers and the racemic mixture of pyriproxyfen were obtained from Takarazuka Research Center, Sumitomo, Hyogo, Japan. The mobile phase tested were composed of UV grade *n*-hexane and various alcohols, which were purchased from Wako (Osaka, Japan). The deuterated solvents for nuclear Overhauser effect (NOE) difference experiments were bought from Aldrich (Milwaukee, WI, USA).

Chromatographic conditions

The solute was dissolved in *n*-hexane–2-propanol (1:1) at a concentration of 1 mg/ml and injected onto the column via a 20-μl loop (Rheodyne injector). The injection volume was 5 μl. The column was operated at ambient temperature and solutes were detected at 254 nm. A flow-rate of 1 ml/min was maintained throughout the study.

Order of enantiomeric elution

To determine the order of elution of the (*S*)- and (*R*)-isomers, a 3:1 mixture [(*S*):(*R*)] of the two isomers was chromatographed. The mixture was prepared by using known amounts of the pure (*S*)-enantiomer and the racemic mixture.

NOE experiments

The $^1H\{^1H\}$ NOE difference spectra were recorded on a JEOL (Tokyo, Japan) JNM-GSX 270J spectrometer with tetramethylsilane as an internal standard. NOE experiments were performed with the irradiation of all the protons of pyriproxyfen in both [$^2H_{14}$]*n*-hexane–[$^2H_8$]2-propanol (9:1) and [$^2H_{14}$]*n*-hexane–[$^2H_{10}$]1-butanol (9:1).

Some connectivities established by NOE difference experiments in both *n*-hexane–2-propanol (9:1) and *n*-hexane–1-butanol (9:1) are listed in Table I.

Circular dichroism spectra

Circular dichroism (CD) measurements were carried out with a JASCO (Tokyo, Japan) J-500 spectrometer, using a 10-mm pathlength cell thermostatted at 23°C. The CD spectra of (*S*)-(−)-pyriproxyfen (4.2 mg/ml) in both *n*-hexane–2-propanol (9:1) and *n*-hexane–1-butanol (9:1) were recorded from 350 to 200 nm. The CD spectra obtained in both solvent systems were almost the same as those of the solvent blank.

TABLE I

RESULTS OF NOE DIFFERENCE EXPERIMENTS

Proton(s) irradiated	Proton(s) affected
1-CH_3 (δ 1.48)	3-H, 2-H
2-H (δ 4.10)	1-CH_3, 3-H, 6,7,8-H
3-H (δ 5.61)	1-CH_3, 2-H
4-H (δ 6.74)	11-H
5-H (δ 6.85)	–
6,7,8-H (δ 6.96)	2-H, 10-H
9-H (δ 7.04)	–
10-H (δ 7.29)	6,7,8-H
11-H (δ 7.55)	4-H
12-H (δ 8.15)	–

RESULTS AND DISCUSSION

The chromatographic resolution of the optical isomer of pyriproxyfen is shown in Fig. 2, using a Chiralcel OJ column and *n*-hexane–1-hexanol (9:1) as eluent. The column was the only one that gave excellent separation of the enantiomers. No resolution was observed using a variety of modified cellulose columns, such a Chiralcel OB, OC, OD, OF, OK or OG. The capacity factors (k'_1 and k'_2) were

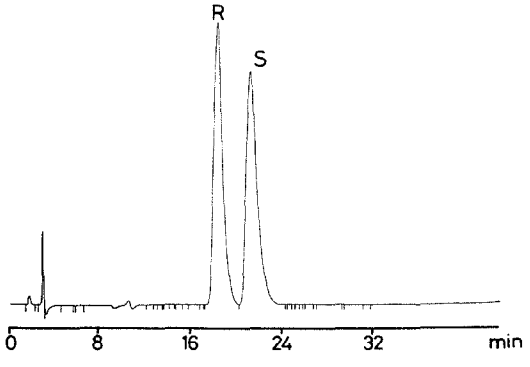

Fig. 2. Enantioseparation profile of racemic pyriproxyfen. Eluent, *n*-hexane–1-hexanol (9:1); for other analytical conditions, see text.

5.15 and 6.18, respectively. The separation factor (α) and the resolution (R_s) were determined to be 1.20 and 1.80, respectively.

We examined the mobile phase effects on the retention and stereoselectivity. A variety of the alcohols were used as the mobile phase modifiers. In general, mobile phases containing 10% alcohol were used, except for methanol and ethanol as with 10% methanol or ethanol the enantiomers were eluted too fast. The separation factor decreased with an increase in the methanol or ethanol concentration, but the elution order did not change. The chromatographic results are presented in Table II. No clear relationship between the capacity factors and the molecular weight and steric bulk of the mobile phase modifier could be identified. Measurable separation was observed in all cases, except for *n*-hexane–2-butanol. This observation does not appear to be affected by the chirality of 2-butanol, because pyriproxyfen could not be resolved using (*S*)-(+)-2-butanol as the mobile phase modifier, and the observed k' was identical with that obtained using the racemic alcohol. The maximum resolution was obtained with 1-hexanol as the mobile phase modifier (α = 1.20), and the minimum resolution with ethanol (α = 1.05).

During this study, we found a unique reversal of elution order on changing the mobile phase modifiers, as shown Table II. Two explanations for this abnormal behaviour are possible: (i) the solvation or the conformation of either pyriproxyfen or the chiral stationary phase is affected by the change in the nature of the modifier; (ii) an alteration in the steric environment of chiral cavity on the stationary phase could be induced by changing the modifier.

We performed extensive NOE and CD experiments to confirm whether a conformational change of the solute occurred depending on the mobile phase used. The $^1H\{^1H\}$ NOE difference and CD spectra of pyriproxyfen in *n*-hexane–2-propanol (9:1) were identical with those in *n*-hexane–1-butanol (9:1). These results show that the conformation of pyriproxyfen did not alter during the separation process. The conformation of the chiral stationary phase (cellulose) is not likely to be changed easily since it is supposed to be rigid [11]. The possibility that solvation occurs could not be ruled out, but Wainer *et al.* [12] have reported that solvation may play only a minor role in the chromatographic process on the cellulose ester derivative chiral stationary phase. Thus we consider that alteration of the conformation of both the solute and the chiral stationary phase does not play an important part in the inversion with a fair degree of certainty.

The observed reversal in the enantiomeric elution order can be explained by the difference of the steric bulk around the hydroxyl moiety of the mobile phase modifier. The enantiomeric elution order was

TABLE II

CHROMATOGRAPHIC RESULTS OBTAINED ON CHIRALCEL OJ

Mobile phase: *n*-hexane–alcohol (v/v).

Alcohol in mobile phase	k'_1	α	R_s	Elution order
Methanol (95:5)	2.53	1.14	1.41	*S*/*R*
Ethanol (95:5)	3.99	1.05	0.48	*S*/*R*
1-Propanol (9:1)	3.80	1.08	0.75	*R*/*S*
2-Propanol (9:1)	3.36	1.13	1.14	*S*/*R*
1-Butanol (9:1)	4.67	1.10	0.98	*R*/*S*
2-Butanol (9:1)	4.16	1.00	–	–
(*S*)-(+)-2-Butanol (9:1)	4.16	1.00	–	–
Isobutanol (9:1)	4.83	1.09	0.83	*R*/*S*
tert.-Butanol (9:1)	6.01	1.06	0.44	*S*/*R*
1-Pentanol (9:1)	5.67	1.10	1.00	*R*/*S*
2-Pentanol (9:1)	4.92	1.10	0.91	*R*/*S*
3-Pentanol (9:1)	4.64	1.06	0.40	*R*/*S*
1-Hexanol (9:1)	5.15	1.20	1.80	*R*/*S*
2-Hexanol (9:1)	6.51	1.19	1.91	*R*/*S*

S/*R* with 2-propanol as the mobile phase modifier, and the elution order was *R*/*S* with 1-propanol, although the solvent polarity parameter (p') values for 1- and 2-propanol are virtually the same, 4.0 and 3.9, respectively [13]. When methanol, ethanol, 2-propanol, or *tert.*-butanol was used as the mobile phase modifier, the (*S*)-(−)-enantiomer eluted before the (*R*)-(+)-enantiomer. The (*R*)-(+)-isomer eluted first with 1-propanol, 1-butanol, isobutanol, pentanols or hexanols. This shows that diastereomeric complex(es) between the chiral stationary phase and the (*R*)-isomer may be more stable with less bulky alcohols, methanol, ethanol, 2-propanol, or *tert.*-butanol, as the mobile phase modifier, and that with more bulky alcohols, 1-propanol, 1-butanol, isobutanol, pentanols, or hexanols, diastereomeric complex(es) between the chiral stationary phase and the (*S*)-isomer may be more stabilized.

It has been reported that the chiral cavity (or ravine) of the stationary phase plays an important role in the chiral recognition on cellulose-based chiral stationary phase [12]. The less bulky alcohols could be inserted into the cavity of the chiral stationary phase more easily than the more bulky alcohols. The insertion of the mobile phase modifier into the chiral cavities of the chiral stationary phase could induce changes in the dominant chiral recognition mechanism, leading eventually to inversion of the elution order of enantiomers. The observation noted above suggests that at least two chiral binding- or recognition-sites are present in this chiral stationary phase.

In this instance, the enantioselectivity and enantiomeric elution order of pyriproxyfen on a cellulose tris-(4-methylbenzoate) depend on the mobile phase modifier used. It will be essential to take into account the contribution of mobile phase modifier to the enantioseparation of racemic compounds.

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