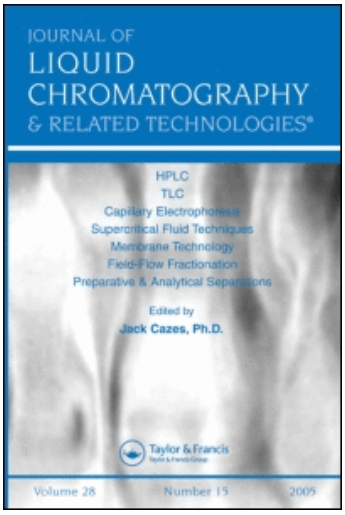

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Preparation of Polysaccharide-Based Chiral Stationary Phases and the Direct Separation of Six Chiral Pesticides and Related Intermediates

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ABSTRACT

Three polysaccharide-based chiral stationary phases (CSPs), including cellulose-*tris*-(3,5-dimethylphenylcarbamate) (CDMPC), cellulose-*tris*-(phenylcarbamate) (CMPC), and amylose-*tris*-(3,5-dimethylphenylcarbamate) (ATPC) were synthesized. The successful resolution of six pesticides and related intermediates on these CSPs, by applying *n*-hexane : isopropyl alcohol mobile phase, is described. The influence of isopropyl alcohol concentration in mobile phase was also studied.

Key Words: Polysaccharide CSPs; Chiral pesticides; Effect of i-PrOH.

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INTRODUCTION

Chirality plays an important role in chemical synthesis, toxicology, environmental control, and design of pharmaceutical agents and pesticides. Among optical enantiomers of chiral compounds, their bioactivities and metabolism mechanisms are usually greatly different from each other.[1]

In the pesticide industry, many of the most widely prescribed pesticides have optical isomers. At present, there are approximately 650 pesticides on the market, among which about 170 pesticides have optical enantiomers, but a large percentage is produced and marketed in the form of racemic mixtures. Often, an enantiomer of a pesticide is biologically active, whereas the other enantiomer may be totally useless, or even toxic to crops. One is environmental friendly, while the other is a serious environmental pollutant which is hard to degrade. The trend to use individual optical enantiomers has given rise to the research for new and effective means to resolve enantiomeric mixtures. The great economic and scientific values make it meaningful to establish methods for separating and determining the optical purity of chiral pesticides and related intermediates.

In the past two decades, chromatographic enantioseparations, particularly direct separation of enantiomers by high-performance liquid chromatography (HPLC), have advanced markedly and this resolution procedure has become one of the most useful methods in many fields dealing with drugs, natural products, agrochemicals, etc., not only for determining their optical purity, but also for obtaining optical isomers on a large scale.[2] The design and development of a chiral stationary phase (CSP) capable of effective chiral recognition of a wide range of enantiomers is the key point of the HPLC technique.[3] A number of CSPs for HPLC have been prepared and more than 100 CSPs have been commercialized.[4] Polysaccharides, mainly including cellulose and amylase, are among the most abundant optically active biopolymers with perfectly defined structures. Among the many types of CSPs for HPLC, polysaccharide-based CSPs showed good chiral recognition ability towards a wide number of different racemic compounds.[5]

Six chiral pesticides and related intermediates were selected which included metalaxyl, quizalofop, metalaxyl intermediate, 2-allyl-4-hydroxy-3-methyl-2-cyclopent-1-one, hexaconazole, and bioallethrin, to investigate the enantiomeric separation of the CSPs synthesized. Every sample was resolved on the three CSPs by using *n*-hexane : isopropyl alcohol as mobile phase. In the first step of our work, we focused on the optimization of analytical conditions to achieve a higher resolution in a shorter analysis time. Each racemate of the six samples obtained good resolution on at least one of the

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three CSPs under different conditions. The effect of isopropyl alcohol concentration in mobile phase on the stereoselectivity was studied.

The successful resolution of the samples on the corresponding CSP has never been reported before.

EXPERIMENTAL

Reagents

Phenylisocyanate and 3,5-dimethylphenylisocyanate were purchased from Merck. Amylose beads were obtained from Sigma, microcrystalline cellulose from ShangHai Fourth Chemicals Reagent Plant (China), and 3-aminopropyl-triethoxysilane (KH-550) from LiaoNing GaiXian Chemicals Plant. Micro spherical silica was made by ourselves with the following properties: particle size, 5–7 μm; average pore diameter, 6.7 nm; specific surface area, 110 m^2/g. Racemic samples were provided by the Institute for Control of Agrichemicals Ministry of Agriculture, and the chemical structures are shown in Fig. 1. All eluents were of analytical grade.

Apparatus

Chromatography was performed using Agilent 1100 Series HPLC equipped with G1311A pump, G1322A degasser, G1328A injector, a 10 μL sample loop, and G1314A VWD. The signal was acquired and processed by an HP1100 workstation. The following parameters were measured:

K_1': capacity factor of the first eluted enantiomer.

K_2': capacity factor of the second eluted enantiomer.

α: selectivity factor.

Rs: resolution factor.

Chromatographic Conditions

The columns were 150 mm × 4.6 mm. Chromatographic separations were performed under room temperature, and eluents were mixtures of appropriate percentages of isopropyl alcohol in *n*-hexane. Injection volume is 10 μL.

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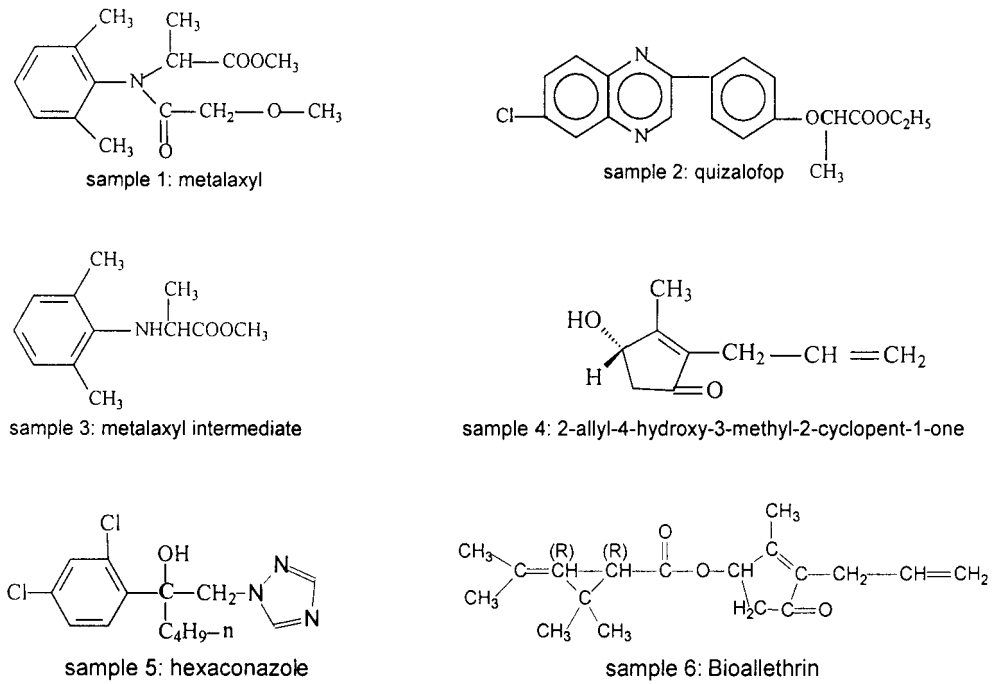

Figure 1. Chemical structures of the molecules under investigation.

Preparation of Chiral Stationary Phases

Amylase/macrocrystalline cellulose (1.0 g) was dissolved in pyridine,[6,7] refluxed for 12 h, 3,5-dimethylphenylisocyanate/phenylisocyanate (3.5 g) was added, then the mixture was refluxed for 48 h. After cooling, methanol (30 mL) was added. The precipitate was filtered, washed with methanol twice, and dried under vacuum for 24 h. Infrared spectra showed that the absorption of hydroxyl groups (3340 cm^{-1}) disappeared.

Spherical silica reacted with 3-aminopropyltriethoxysilane in toluene at 110°C for 24 h; the product, aminopropylsilica (APS), was filtered off and washed with toluene and dried at 80°C under vacuum. Cellulose-*tris*-(3,5-dimethylphenylcarbamate) (CDMPC)/cellulose-*tris*-(phenyl-carbamate) (CMPC)/amylose-*tris*-(3,5-dimethylphenylcarbamate) (ATPC) (0.45 g) was dissolved in 30 mL tetrahydrofuran, APS (2.55 g) was added. The mixture was stirred for 15 min, after evaporating solvent, and dried at 60°C for 8 h under vacuum.

Preparation of Chiral Column

The slurry of CSPs in hexane-isopropyl alcohol (90 : 10, v/v) solution was packed into a stainless steel column under 3.7×10^7 Pa pressure.

RESULTS AND DISCUSSION

The racemate of sample 1 yielded good resolution on the CDMPC CSPs. Table 1 lists the chromatographic parameters for the influence of isopropyl

Table 1. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 1.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
15	8.34	16.50	1.98	5.32
20	5.72	10.63	1.86	4.54
25	4.41	7.76	1.76	3.85
30	3.08	5.09	1.65	3.52
35	2.94	4.45	1.51	2.70
40	2.26	3.01	1.33	1.24
45	2.04	2.53	1.24	1.08

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alcohol on the resolution. Cellulose-*tris*-(phenylcarbamate) and ATPC present no stereoselectivity. The racemic mixture of sample 2 obtained good resolution on the ATPC CSP only. The influence of isopropyl alcohol on the separation is shown in Table 2. The racemate of sample 3 obtained good resolution on the CDMPC CSP. Table 3 shows the influence of isopropyl alcohol on the selectivity. Amylose-*tris*-(3,5-dimethylphenylcarbamate) and CMPC showed no stereoselectivity. The two enantiomers of sample 4 were separated on the CDMPC CSP only. Table 4 shows the influence of isopropyl alcohol on the

Table 2. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 2.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
0	23.98	28.94	1.21	1.22
0.5	9.54	11.02	1.16	1.05
2	8.39	8.73	1.04	0.84
10	4.42	4.42	1.00	0

Table 3. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 3.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
0	23.98	28.94	1.21	1.22
0.5	9.54	11.02	1.16	1.05
2	8.39	8.73	1.04	0.84
10	4.42	4.42	1.00	0

Table 4. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 4.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
1	40.86	54.55	1.34	1.36
2	19.05	23.81	1.25	0.96
5	5.51	6.56	1.19	0.66
10	1.89	2.22	1.17	0.17
20	0.62	0.62	1.00	0

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selectivity. The racemates of sample 5 were separated on the CDMPC CSP only. Table 5 shows the influence of isopropyl alcohol on the separation.

The two enantiomers of bioallethrin were separated both on the CDMPC and on the CMPC CSPs. Tables 6 and 7 show the influence of isopropyl

Table 5. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 5.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
2	no elution			
5	10.47	17.23	1.65	1.79
10	4.65	7.49	1.61	1.70
15	3.13	5.74	1.83	1.54
20	2.29	3.36	1.47	1.44

Table 6. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 6 on CDMPC CSP.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
1	4.11	5.22	1.27	1.40
5	1.64	1.91	1.16	1.21
10	1.22	1.37	1.12	1.03
15	1.01	1.10	1.09	0.96
20	0.89	0.96	1.08	0.88

Table 7. The effect of isopropyl alcohol volume percent in mobile phase on the resolution of sample 6 on CMPC CSP.

Isopropyl alcohol volume (%)	K_1'	K_2'	α	*Rs*
1	4.11	5.62	1.37	1.61
5	1.29	1.74	1.35	1.45
10	0.68	0.92	1.35	1.41
15	0.39	0.50	1.28	1.29
20	0.34	0.43	1.26	1.15

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alcohol on the selectivity. Amylose-*tris*-(3,5-dimethylphenylcarbamate) showed no stereoselectivity to the sample.

The results showed that CDMPC was appropriate for the resolution of samples 1, 3, 4, 5, and 6. When the percentage of isopropyl alcohol in mobile phase decreased, the selectivity factor increased, while it resulted in a longer retention time for all the above samples. Amylose-*tris*-(3,5-dimethylphenylcarbamate) presented high enantioselectivity for sample 2 only, and CMPC showed good enantioselectivity for sample 6 only; the influence of isopropyl alcohol on the resolution was the same as above. We have established ideal methods for the separation of the samples.

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