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Short Communication

Enantiomeric separation of racemic thiosulphinate esters by high-performance liquid chromatography

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ABSTRACT

High-performance liquid chromatographic methods using chiral stationary phases were developed for the separation of racemic mixtures of biologically active alk(en)ylsulphinothioic acid alk(en)yl esters (syn. thiosulphinate esters, ts) from natural (*Allium cepa* L.) or synthetic origin. Aromatic substituted thiosulfinate esters could be baseline resolved using helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel [Chiralpak OT (+)] as chiral stationary phase and methanol as eluent. A correlation between chromatographic resolution and the structures of the thiosulphinate esters could be established. A preparative separation of diphenyl-ts was achieved with cellulose triacetate (CTA) as the stationary phase. The elution sequence of diphenyl-ts enantiomers on the CTA column is reversed when compared to that on the Chiralpak OT (+).

INTRODUCTION

The use of high-performance liquid chromatography (HPLC) for the separation of racemates is increasing rapidly [1–4], especially owing to the often observed different biological activities of the enantiomers [5].

Chiral alk(en)ylsulphinothioic acid alk(en)yl esters (syn. thiosulphinate esters, ts) have recently been described as anti-asthmatic active constituents of onion juice [6] and as potent dual *in vitro* inhibitors of 5-lipoxygenase and cyclooxygenase [7]. They have also shown an inhibitory effect on platelet aggregation [8].

Thiosulphinate esters in Allium extracts (*Allium cepa* L. or *Allium sativum* L.) occur as racemic mixtures; stereoselective synthesis of enantiomerically pure thiosulphinate esters has succeeded in only a few cases [9]. Therefore, a suitable method for

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the separation of thiosulphinate ester racemates is needed in order to study the biological effects of pure enantiomers.

In this paper we describe HPLC methods for the separation of racemic thiosulphinate esters using helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel and microcrystalline cellulose triacetate (CTA) as chiral stationary phases.

EXPERIMENTAL

Synthesis of thiosulphinate esters

Methyl phenyl disulphide and allyl phenyl disulphide were prepared by disproportionation of a symmetrical disulphide, dimethyl disulphide and diallyl disulphide, respectively, and thiophenol in alkaline solution [10]. These non-symmetrical disulphides and dimethyl, diallyl, diphenyl and ditolyl disulphide (obtained from Merck, Darmstadt, Germany) were oxidized by 3-chloroperbenzoic acid according to the method of Small *et al.* [11]. The corresponding thiosulphinate esters were purified by flash chromatography or medium-pressure LC [12].

HPLC procedure

The HPLC system consisted of a Hewlett-Packard (HP) 1090A liquid chromatograph, an HP 1040A photodiode-array detector, an HP 3392A integrator and an HP 7470 plotter. Aliphatic thiosulphinate esters were detected at 210 nm and aromatic esters at 280 nm. Polarimetric detection was performed using an ACS ChiraMonitor (Applied Chromatography Systems, Cheshire, U.K.) with a 20-μl flow cell of path length 20 mm using a 2-mW collimated near-IR laser diode (830 nm) as light source.

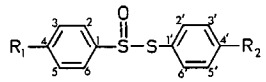
The HPLC separation of racemic thiosulphinate esters was performed with a (+)-poly(triphenylmethyl methacrylate)-coated silica gel column [Chiralpak OT (+), 250 mm × 4.6 mm I.D.; Daicel] with methanol as eluent at a flow-rate of 0.5 ml/min. For preparative enantiomeric separations we used a Hibar cellulose triacetate (10 μm) column (250 mm × 10 mm I.D.) (Merck) eluted with ethanol. All solvents were of HPLC grade (Promochem, Wesel, Germany). The separations were performed at 20 ± 1°C. The injection volume was 3 μl of a 0.1% solution of thiosulphinate esters in ethanol; when using the ACS ChiraMonitor the injection volume was 10–30 μl.

RESULTS AND DISCUSSION

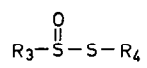
Enantiomeric separation of racemic thiosulphinate derivatives **1**–**11** (Fig. 1) was performed by HPLC using helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel as chiral stationary phase and methanol as eluent. Five racemic thiosulphinate esters, **1**–**3**, **5** and **6**, were baseline resolved ($R_s > 1$). Their chromatographic data are shown in Table I and Fig. 2.

Polarimetric detection showed that all dextrorotary enantiomers were eluted first from the column (Fig. 3). This type of detection was *ca.* 100 times less sensitive than UV detection.

The highest chromatographic resolution ($R_s = 3.75$) and separation factor ($\alpha = 1.31$) were found for the enantiomers of phenylsulphinothioic acid S-phenyl ester (diphenyl-ts) **1**. Introduction of a methoxy group in the 4-position (**4**) decreased the resolution drastically ($R_s = 1.01$). This negative effect could be compensated for by



	R_1	R_2
1	H	H
2	CH_3	CH_3
3	H	OCH_3
4	OCH_3	H
5	OCH_3	OCH_3
6	Cl	H



	R_3	R_4
7	C_6H_5	CH_3
8	CH_3	C_6H_5
9	C_3H_5	C_6H_5
10	C_3H_5	C_3H_5
11	CH_3	CH_3

Fig. 1. Structures of compounds.

a further *p*-methoxy substituent in the second aromatic ring, as in **5** (R_s = 1.52), while the chromatographic resolution of the enantiomers of phenylsulphinothioic acid S-*p*-methoxyphenyl ester **3** (R_s = 3.22, α = 1.27) was similar to that of diphenyl-ts **1**. Good resolutions were also observed for ditolyl-ts **2** (R_s = 1.52) and *p*-chlorophenylsulphinothioic acid S-phenyl ester **6** (R_s = 2.05). Therefore, the resolution of diphenyl-ts enantiomers seems to be negatively influenced especially by substituents in the *para* position to the sulphoxide, whereas substituents in the 4'-position have less effect.

The enantiomers of dimethyl-ts **11** and dially-ts **10**, which also occur in extracts of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.), respectively, could not be resolved by the described separation system. Also, synthetic enantiomers of non-symmetrically substituted thiosulphinate esters, such as methylsulphinothioic acid S-phenyl ester (methyl phenyl-ts) **8** and allylsulphinothioic acid S-phenyl ester (allyl phenyl-ts) **9**, both containing an aliphatic sulphinothioic acid moiety, but an aromatic ester moiety, were not resolved. However, optical resolution was achieved for the enantiomers of phenylsulphinothioic acid S-methyl ester (phenyl methyl-ts) **7**. These results clearly showed that an aromatic sulphinothioic acid moiety is necessary for the

TABLE I

CHROMATOGRAPHIC DATA FOR RACEMIC THIOSULPHINATES

Stationary phase: (+)-poly(triphenylmethyl methacrylate)-coated silica gel. Mobile phase: methanol (0.5 ml/min). t_R = Retention time; R_s = resolution factor = 1.18 × (distance between the peaks of the enantiomers)/(sum of band widths of the two peaks at the peak half-height); k' = capacity factor = (retained volume of enantiomer − void volume of column)/void volume of column; α = separation factor = $k'(-)/k'(+)$.

ts	$t_R(+)$ (min)	$k'(+)$	$t_R(-)$ (min)	$k'(-)$	α	R_s
1	13.19	2.56	16.11	3.35	1.31	3.75
2	11.06	1.99	12.22	2.30	1.16	1.52
3	12.32	2.33	14.66	2.96	1.27	3.22
4	12.56	2.39	13.51	2.65	1.11	1.01
5	11.97	2.24	13.16	2.56	1.14	1.52
6	12.26	2.31	14.84	3.01	1.30	2.05

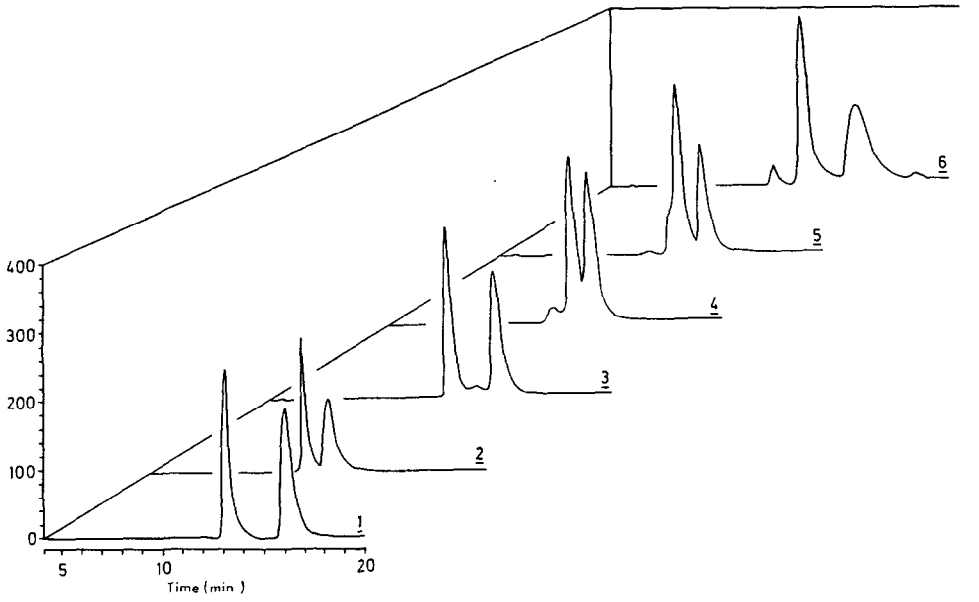


Fig. 2. HPLC separations of ts **1**–**6** on (+)-poly(triphenylmethyl methacrylate)-coated silica gel. Mobile phase: methanol at a flow-rate of 0.5 ml/min. Photometric detection at 280 nm.

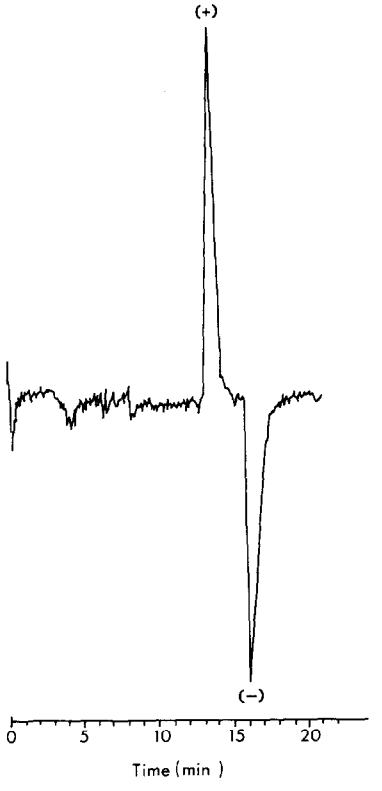


Fig. 3. HPLC separation of diphenyl-ts on (+)-poly(triphenylmethyl methacrylate)-coated silica gel. Mobile phase: methanol at a flow-rate of 0.5 ml/min; ACS ChiraMonitor.

separability of thiosulphinate esters by helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel. This again supports the assumption that an interaction between the aromatic part of the molecules and the pendant trityl groups of the polymeric chain is an important factor for enantiomeric separations on this type of stationary phase [13].

For the preparative separation of diphenyl-ts **1** we used cellulose triacetate (CTA) as stationary phase. Of various alcohols, *e.g.*, methanol (R_s = 0.64) and isopropanol (R_s = 0.59), ethanol (R_s = 1.11) was found to be the best mobile phase. By using this method we could isolate the enantiomers of diphenylthiosulphinate ester for the first time.

Testing the purity of the isolates by HPLC using helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel we observed that the isolated peaks were enantiomerically pure. It also became obvious that the elution sequence of diphenyl-ts enantiomers from the CTA column was reversed compared with the separation using helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel.

Before starting investigations on the biological activities, the stability of isolated diphenyl-ts enantiomers must be examined. A possible racemization of the enantiomers during biological assay methods can now be monitored by the established HPLC method using helical poly(triphenylmethyl methacrylate)-coated silica gel as chiral stationary phase and methanol as eluent.

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