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Note

Direct liquid chromatographic separation of enantiomers on immobilized protein stationary phases

II*. Optical resolution of a sulphoxide, a sulphoximine and a benzoylamino acid

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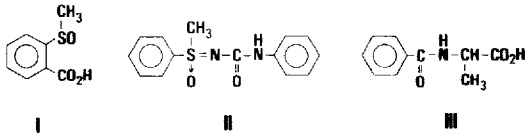
In the previous paper in this series successful direct resolutions of some racemic aromatic amino acids into enantiomers by means of isocratic liquid affinity chromatography on albumin–agarose were reported[1]. The potential use of this method might be predicted from the few literature data on enantioselective binding to serum albumins[2]. Most of these data were obtained from studies of equilibria between albumins and diverse racemic compounds, but quite recently a chromatographic technique based on the use of immobilized albumin was also utilized[3,4]. A short review, covering some aspects on the chiral discrimination exerted by serum albumin for direct resolution in liquid affinity chromatography, was presented recently[5].

In this paper the importance of this chromatographic method for the direct separation of optical antipodes is further emphasized by its successful application to new types of compounds and by a demonstration of the large separation factors that may be achieved by various means of regulating the retention of the enantiomers. The enantioselectivity exerted by the serum albumin stationary phase towards two chiral sulphur compounds, resulting in complete resolution under appropriate conditions, is demonstrated for the first time. Further, N-benzoylated α-amino acids appear to be resolvable, as shown for N-benzoyl-D,L-α-alanine. The structures of these compounds are shown in Fig. 1.



I II III

Fig. 1. Structures of the compounds shown to be resolvable on albumin–agarose.

* Ref. 1 is regarded as Part I of this series.

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EXPERIMENTAL

Chromatography

The BSA-agarose was synthesized from activated CH-Sepharose 4B (Pharmacia) as described previously[1]. The gel was packed into a 6.6 × 150 mm glass column (Omnifit, Cambridge, Great Britain), which was connected to a high-performance liquid chromatography (HPLC) system comprised of an Altex Model 110A solvent pump, a Rheodyne Model 7120 injection valve with a 10-μl loop, an LDC Spectromonitor III variable-wavelength UV detector and a Hitachi Model 561 potentiometric recorder. Phosphate buffers (5–200 mM) were used as the mobile phase at a flow-rate of 1.0 ml/min unless stated otherwise. Solutions of the compounds dissolved in the mobile phase were injected.

Compounds

2-Methylsulphinylbenzoic acid (I) was prepared as described elsewhere[6,7]. The pure enantiomers of I were obtained via a classical resolution procedure using brucine as the resolving base[6,7]. Recrystallizations from water yielded *R*-(+)-I, m.p. 183–184°C, $[\alpha]_{546}^{20} = +289.7$ (ethanol, c 0.8), and *S*-(−)-I, m.p. 182–183°C, $[\alpha]_{546}^{20} = -274.0$ (ethanol, c 1.1).

Methyl 2-methylsulphinylbenzoate (Ia) was prepared by esterification of I with diazomethane in methanol[8], m.p. 65°C.

N-Phenylcarbamyl-S-methyl-S-phenyl-sulphoximine (II) was synthesized via reaction of S-methyl-S-phenyl-sulphoximine, prepared according to Johnson *et al.*[9], with phenyl isocyanate[8,9], m.p. 125–128°C (129–130°C[9]).

N-Benzoyl-α-alanine (III) was obtained from ICN Ltd.

Determination of optical purity

Polarimetric measurements were carried out with a Perkin-Elmer Model 243B photoelectric polarimeter at 546 nm using a 1-ml quartz microcell of 10 cm pathlength. Chromatograms of *R*-(+)-I showed no traces of the opposite enantiomer, whereas 2% of *R*-(+)-I as a contaminant could be evaluated from chromatograms of *S*-(−)-I. Polarimetric data give an optical purity of 95% for *S*-(−)-I.

RESULTS AND DISCUSSION

The chromatographic behaviour of *rac*-I as well as the pure optically active forms on the albumin–agarose column is shown in Fig. 2. In an attempt to find the optimal conditions for separation of the enantiomers of I, a study of the influence of the mobile phase composition on the k' values and α was performed (Fig. 3). In contrast to the behaviour of the amino acids studied previously[1], where the k' values were found to increase with increasing pH of the buffer, the opposite was true for I as shown in Fig. 3a. Unfortunately, the region below pH 5.5 was inaccessible without a change of the buffer agent. However, a general trend towards larger α values with decreasing pH is evident. Besides pH the ionic strength is another important factor of the mobile phase and the effect of lowering the buffer concentration at a constant pH is shown in Fig. 3b. The k' values increase very rapidly below 50 mM buffer concentration and the effect is larger for the more strongly retained *R*-(+)-enantiomer, which leads to a progressive increase of the α value.

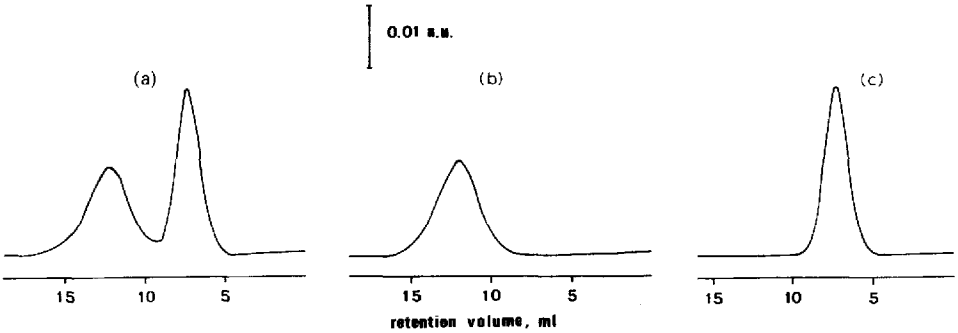

Fig. 2. Chromatograms of *rac.*-I (a), *R*-(+)-I (b) and *S*-(−)-I (c), respectively, under identical conditions. Mobile phase: 0.20 *M* phosphate buffer (pH 6.45), flow-rate 0.80 ml/min. UV detection at 235 nm; 0.1 a.u.f.s. Sample volume injected, 10 μl. Concentrations: 0.41 m*M* (a); 0.24 m*M* (b); 0.23 m*M* (c).

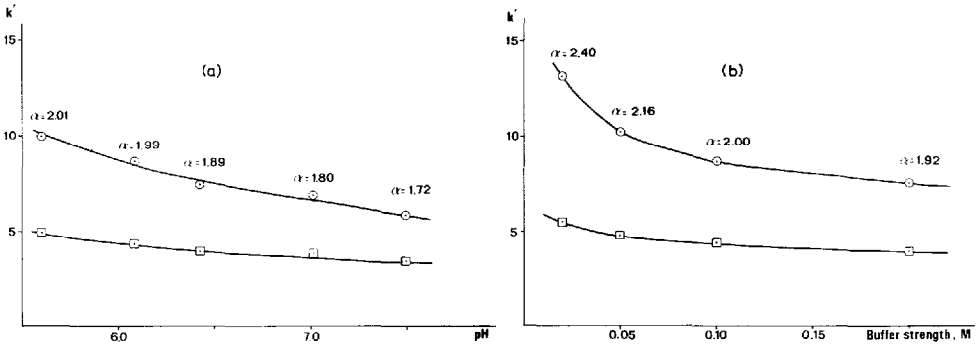

Fig. 3. The capacity factors, *k'*, of the enantiomers of I as a function of the mobile phase composition. Influence of pH (a) and the buffer strength (b). Phopshate buffers: 0.1 *M* (a); pH 6.5 (b); flow-rate 1.0 ml/min. Sample: 0.41 m*M* *rac.*-I, 10 μl injected. UV detection at 235 nm; 0.1 a.u.f.s. ⊙, *R*-(+)-I; ⊡, *S*-(−)-I.

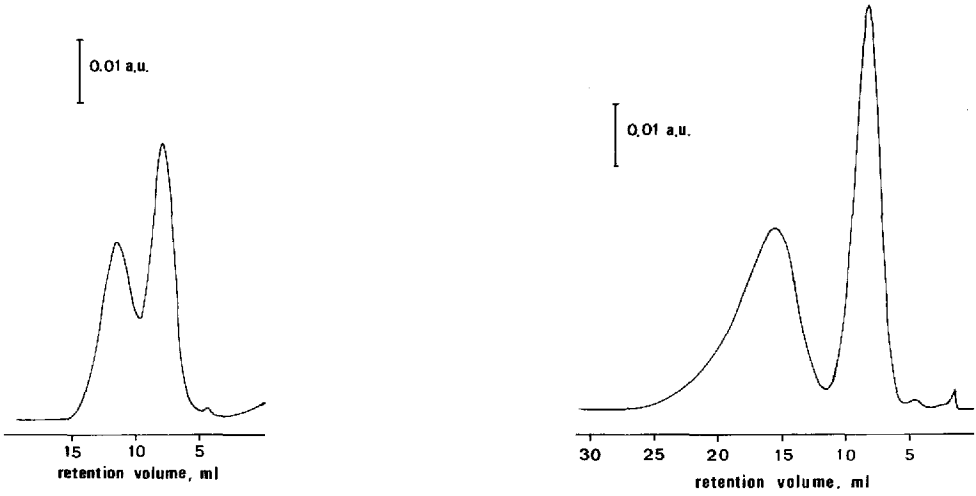

Fig. 4. Partial separation of the enantiomers of *rac.*-II. Mobile phase: 5 m*M* phosphate buffer (pII 5.6), flow-rate 1.0 ml/min. UV detection at 235 nm; 0.1 a.u.f.s. Sample: 0.5 m*M*, 10 μl injected.

Fig. 5. Chromatogram showing the complete resolution of *rac*-III. Mobile phase: 10 m*M* phosphate buffer (pH 6.64), flow-rate 1.0 ml/min. UV detection at 235 nm; 0.1 a.u.f.s. Sample: 1.35 m*M*, 10 μl injected.

The carboxyl group in I appears to be essential for chiral recognition and resolution of the two enantiomers. Thus, the racemic methyl ester Ia is almost unretained and does not show any sign of resolution.

Due to the short column length used in this study and the relatively low efficiency in terms of plate height an α value of >2.0 is required for a complete baseline separation of the antipodes. Much more efficient columns, however, can be made on binding the protein to wide-pore HPLC silica[5]. Therefore, we are convinced, especially in the light of these new results, that HPLC on immobilized protein stationary phases will become a powerful analytical tool in the future, particularly for the determination of optical purity at low concentration levels.

The resolving power of the albumin–agarose is further demonstrated for the compounds II (Fig. 4) and III (Fig. 5). No optimization with respect to the resolution of these compounds has yet been made and the order in which the enantiomers elute has not been established.

To the best of our knowledge, all compounds known so far to exhibit an enantioselectivity in their binding to serum albumins have been chiral with respect to a carbon atom[2]. The present findings that chirality at sulphur will cause albumin to discriminate between the antipodes raise new questions concerning binding sites and chiral recognition models.

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