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Note

Chromatographic optical resolution of hydantoins by poly(N^5-benzyl-L-glutamine) covalently bound to polystyrene resin

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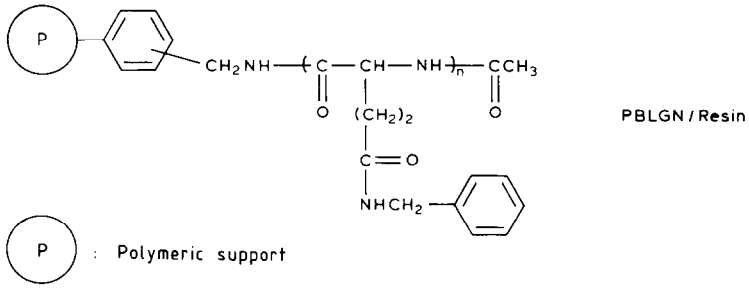
The use of high-performance liquid chromatography for the optical resolution of racemic mixtures has recently increased rapidly and various types of chiral stationary phases are now commercially available[1]. However, almost all of these phases are limited for analytical purposes and only very few can be applied for a preparative scale resolution.

One of the aims of our laboratory is the development of novel chiral adsorbents with functions similar to enzymes, and which withstand the requirements for industrial applications. The highly ordered helical structure of poly(α-amino acids) has attracted our attention for use as novel chiral stationary phases in chromatographic optical resolution. The recognition interaction is assumed to occur between the side chains of the poly(α-amino acid) helix and the stereoisomers by hydrogen bonding. Cross-linked porous polystyrene was chosen, in place of the generally used silica gel, as the support resin.

On this basis, several cross-linked polystyrene resins incorporating poly(L-glutamic acid) derivatives were synthesized and evaluated for the optical resolutionn of enantiomers[2]. Of these adsorbents, immobilized poly(N^5-benzyl-L-glutamine) (PBLGN) has been shown[3] to resolve stereoisomers most efficiently, and a baseline separation of (*RS*)-5-isopropylhydantoin was obtained with 16.2% (w/w) PBLGN, the number-average degree of polymerization, $\overline{DP}$, being 36.



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The aim of the current work is to elucidate the chiral recognition mechanism of the above adsorbent. Experimental evidence so far indicates that the site responsible for the chiral recognition is the amide group in the PBLGN side chain. We describe the elucidation of the shape and dimensions of the chiral recognition site by resolving various hydantoin derivatives with substituents of various bulkiness.

EXPERIMENTAL

Samples

γ-Methyl-L-glutamate (γ-MLG) was obtained from Peptide Institute Inc. (Osaka, Japan). γ-MLG-N-carboxy anhydride (NCA) was prepared by the reaction of γ-MLG with phosgene, as described[4]. Solvents used for the polymerization of amino acid NCA derivatives were dried over molecular sieves 4A before use. (*RS*)-5-Isopropylhydantoin (*RS*-IPH) was prepared from isobutyraldehyde by the Bucherer–Bergs synthesis[5]. Other hydantoin derivatives were synthesized from corresponding aldehydes by similar methods. Commercially available monomers and chemical reagents of special grade purity were used without further purification.

Synthesis of adsorbents

A polystyrene-based adsorbent incorporating 16% (w/w) PBLGN ($\overline{DP}$ = 36) was synthesized by the following procedure. A 9% cross-linked porous polystyrene resin (particle diameter 20–30 μm), incorporating aminomethyl groups, was prepared as a starting material. Utilizing the incorporated primary amine as an initiator, γ-MLG · NCA was polymerized in 1,2-dichloroethane. Elemental analyses confirmed almost quantitative polymerization. The methyl ester of the incorporated poly(γ-MLG) was then converted into the 2-chloroethyl ester by transesterification with 2-chloroethanol using sulphuric acid as a catalyst. The 2-chloroethyl ester thus obtained was then converted into the benzylamide by aminolysis with benzylamine, producing PBLGN-immobilized resin. The terminal amino group of the immobilized PBLGN main chain was blocked by treating the adsorbent with acetic anhydride in dioxane, generating the adsorbent (PBLGN/Resin) for optical resolution. Details of this synthesis have been described[3].

Column packing and liquid chromatography

A Shimadzu LC-4A liquid chromatograph was used for column packing and liquid chromatography. The adsorbent was packed in a stainless-steel column (50 cm × 0.76 cm I.D.) under a constant pressure of 80 atm. Optical resolution was performed at 10°C (in order to enhance hydrogen bonding between the enantiomer and the adsorbent), using a toluene–dioxane mixture as an eluent at a flow-rate of 0.5 ml/min. The sample amount introduced was 1 mg (0.2 ml of 0.5%, w/v solution). The elution curve was monitored by a Shodex SE-51 refractive index (RI) detector and the polarity of the eluates was simultaneously monitored by a JASCO DIP-181C polarimeter detector (365 nm; cell 5 cm × 0.10 cm I.D.).

RESULTS AND DISCUSSION

The results of resolution are summarized in Table I. Separation coefficients,

α, up to 1.78 were obtained, and several baseline separations were accomplished on this adsorbent. Some of the characteristic chromatograms are illustrated in Fig. 1. The *R*(+)-isomers are eluted first for all the hydantoin derivatives that are resolved. For the unresolved compounds, small positive and negative peaks are obtained with the polarimeter detector even though only a single peak is observed with the RI detector. Further, a comparison of the α values of compounds 1a, 1b and 1c indicates a rise in value with increasing bulkiness of R_3 (1.26 < 1.51 < 1.78). Here, R_2 = H while R_3 = *n*-propyl, isopropyl or 1-ethylpropyl, respectively. Additionally, compounds such as 1d and 1e are also resolved but with larger *k'* values and a smaller α value than expected for compound 1d. This latter result is presumably due to the weak association between the side chains of the enantiomers and PBLGN, offsetting the steric hindrance.

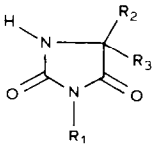
It is noteworthy that compound 2 is not separated while compound 1b is baseline separated, the only difference being the methyl-substituted atom N(3) in compound 2. Since compound 2 is only weakly retained, it is assumed that either this compound is too bulky for the recognition site, or that the hydrogen bonding of N(3) with the amide group in the PBLGN side chain is the main cause of retention. Turning next to compounds with R_2 = methyl, the α values again increase with increasing bulkiness of R_3, as in compounds 3a and 3b (1.59 < 1.76). However, when R_3 = isopropyl, compound 3c experiences almost no retention and thus no separation.

These results demonstrate that the steric difference between the R_2 and R_3 substituents, of hydantoin derivatives, plays an important rôle in determining the chiral selectivity of the stereoisomers. The facts that this adsorbent efficiently recognizes the difference between methyl and ethyl, since compound 3a is baseline separated, and that the methyl- and isopropyl-substituted compound 3c experiences almost no retention, indicate the rigidity and the specific dimensions and shape of the recognition site.

TABLE I

RESOLUTION OF VARIOUS HYDANTOIN DERIVATIVES

For conditions, see Fig. 1. k'_1 = Capacity factor of the first enantiomer eluted = $(t_1 - t_0)/t_0$, where t_1 is the retention time and t_0 is the retention time of an unretained compound (toluene); k'_2 = capacity factor of the second enantiomer eluted. α = Separation coefficient = k'_2/k'_1; (±) = polarity of the first enantiomer eluted. R_s = Resolution factor = 2 · (distance between two peaks)/(sum of bandwidths of two peaks).

General structure	*No.*	R_1	R_2	R_3	k'_1	k'_2	α (±)	R_s
	1a	H	H	*n*-C_3H_7	0.77	0.97	1.26 (+)	0.91
	1b	H	H	*iso*-C_3H_7	0.68	1.02	1.51 (+)	1.43
	1c	H	H	$CH(C_2H_5)_2$	0.43	0.77	1.78 (+)	1.67
	1d	H	H	$(CH_2)_2SCH_3$	1.00	1.17	1.17 (+)	0.54
	1e	H	H	$CH_2C_6H_5$	0.51	0.67	1.33 (+)	0.89
	2	CH_3	H	*iso*-C_3H_7		0.12	– (+)	–
	3a	H	CH_3	C_2H_5	0.47	0.75	1.59 (+)	1.50
	3b	H	CH_3	*n*-C_3H_7	0.42	0.75	1.76 (+)	1.66
	3c	H	CH_3	*iso*-C_3H_7		0.15	– (–)	–

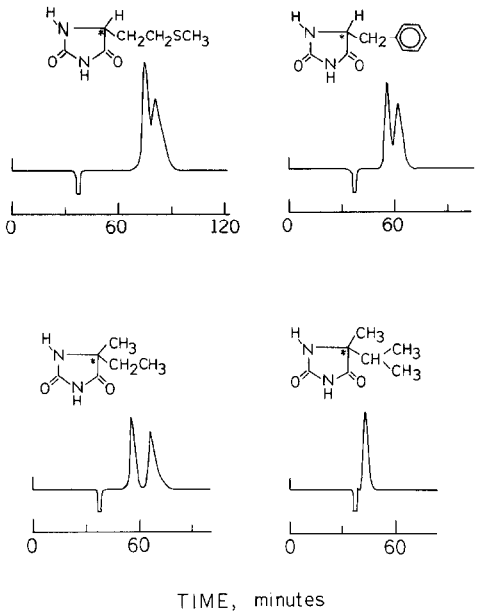


Fig. 1. Chromatograms of compounds 1d, 1e, 3a and 3c. Column: 50 cm × 0.76 cm I.D. Eluent: toluene–dioxane (75:25, v/v); flow-rate 0.5 ml/min. Temperature: 10°C. Adsorbent: 16.2% (w/w) PBLGN, $(\overline{DP})$ = 36. Sample amount: 1 mg (0.2 ml of 0.5%, w/v solution).

In addition, other heterocyclic drugs were evaluated on this adsorbent. It was again interesting to observe that 4-hydroxy-2-cyclopentenone, an intermediate of prostaglandin was partially resolved while other bulkier drugs, such as hexobarbital ($C_{12}H_{16}N_2O_3$) and chlorthalidone ($C_{14}H_{11}ClN_2O_4S$), were hardly retained and thus not resolved.

Work is continuing on the determination of the site of hydrogen bonding between the PBLGN side chain and the enantiomer, and the mechanism with which this adsorbent performs chiral recognition.

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