Chiral Differentiation by the *P*-(+)-Hexahelicene-7,7′-dicarboxylic Acid Disodium Salt. Resolution of *N*-2,4-Dinitrophenyl-α-amino-acid Esters by High Performance Liquid Chromatography

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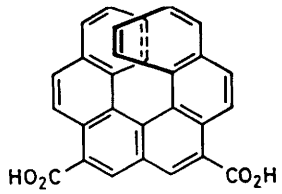
Derivatives of α-amino-acid esters containing a strong electron acceptor group, were resolved on silica gel coated with a chiral electron donor substance.

Charge transfer (C.T.) complexation with chiral molecules incorporated into the stationary phase has been shown to lead to effective resolution of optical isomers.[1–3] We have previously studied silica gel modified by electron-withdrawing reagents, such as *R*-(−)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid [(−)-TAPA][1] and, more recently, riboflavin,[4] nucleosides, and nucleotides.[5]

We report here on the extension of this approach to electron-donating substances, as the chiral, C.T.-complexing component in the stationary phase. Such supports should be of wide interest, as many substances can be made to be strong electron acceptors by forming derivatives with suitable reagents. Obvious examples are the *N*-2,4-dinitrophenyl(Dnp)-α-amino-acid esters, on which the present resolution studies were made.

Helicene derivatives were chosen as the electron-donating substances, as these overcrowded helical molecules showed relatively high chiral differentiation in their interaction with TAPA.[1] Also, Lochmuller and co-workers[6] have reported some measure of enantiomer separation, when chromatographing hexahelicenes on silica gel to which Dnp-L-alanine is bonded.

The particular derivative selected was hexahelicene-7,7′-dicarboxylic acid (**1**), which was resolved with a resolution coefficient (*r*) as high as 2.00 on TAPA.[7] The compound is relatively readily available *via* the corresponding dinitrile, synthesized according to the method of Jutz.[8] (+)-(**1**) was



P-(+)-(1)

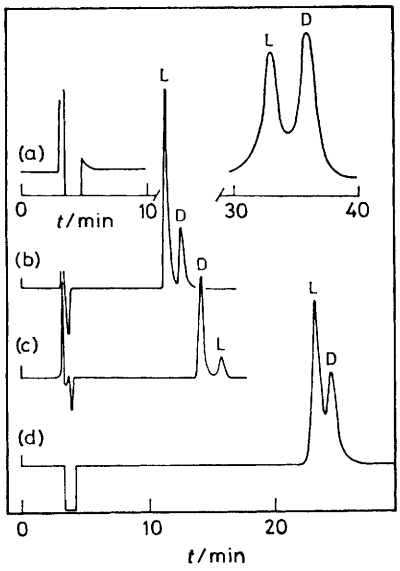


Figure 1. Chromatograms of Dnp-amino-acid methyl esters on the *P*-(+)-hexahelicene-7,7′-dicarboxylic acid disodium salt, coated on silica gel. Derivatives of: (a) alanine; (b) valine (L/D = 3/1); (c) phenylglycine (L/D = 1/3); (d) phenylalanine (L/D = 3/1). See Table 1 for conditions.

obtained[7] in two steps. i, Cinchonidine yielded a precipitate (from EtOH) of pure (+)-(**1**), while from the mother liquor a mixture which was only 50% enriched in the (+)-isomer could be crystallized. ii, The strychnine salt, formed in tetrahydrofuran (THF) from the partially resolved material from i, gave on decomposition 100% optically pure (+)-(**1**), as determined by h.p.l.c.

The chromatographic conditions are summarized in Table 1. Detection was effected at 254 nm. The chiral support was prepared by neutralizing (with 0.3 M NaOH) 100 mg of (+)-(**1**) dissolved in THF : MeOH : H_2O (1 : 1 : 1), and coating on 3 g of Lichrosorb Si 100 (Merck). The silica gel, after evaporation of the solvent and drying, was thoroughly washed with CH_2Cl_2; loading: 3.5% w/w sodium salt of (+)-(**1**).

The compounds examined and the data obtained are given in Table 1 and Figure 1. All the Dnp derivatives listed, except III (Leu), were resolved under the conditions of Table 1. The coefficients *r* were, in general, ≥ 1.1, except for VII, which had an *r* value of only 1.058. In view of the relatively limited experimental material available, the following comments on the influence of structural factors on the chromatographic behaviour should be considered to be tentative.

Except for the Dnp-phenylglycine esters (IX and X), the D-isomer emerged first on (+)-(**1**). This order is in agreement with the stronger interaction of *P*-(+)-helicenes with *R*-(−)-TAPA.[1] Indeed, TAPA and the Dnp-amino-acid esters belong to the same general class of compounds, namely carboxylic acid derivatives with an electron-acceptor group of related structure contained in the substituent at the α-position. Hence,

Table 1. Resolution of Dnp-amino-acid esters, $(NO_2)_2C_6H_3NHCH(R^1)CO_2R^2$, on silica gel coated with *P*-(+)-disodium, hexahelicene-7,7′-dicarboxylate.[a]

No.	Amino-acid $R^1CH(NH_2)CO_2H$	R^2	k_1	k_2	$r_{D/L}$[b]	Eluant % Pr^iOH in n-C_6H_{14}
I	Ala	Me	10.07	11.07	1.099	2
II	Ala	Pr^i	2.23	2.53	1.135	2
III	Leu	Me	2.47	2.47	1.000	1
IV	Ile	Me	2.73	3.20	1.172	1
V	Val	Me	2.83	3.23	1.141	2
VI	Val	Pr^i	1.53	1.73	1.131	1
VII	Phe	Me	6.87	7.27	1.058	2
VIII	Phe	Pr^i	3.27	3.80	1.162	1
					$r_{L/D}$	
IX	Phenylgly	Me	3.80	4.33	1.139	2
X	Phenylgly	Pr^i	1.65	1.95	1.182	1

[a] Chromatographic conditions: column dimensions 21 × 0.46 cm; flow rate 1 ml min^{-1}; room temp. [b] $r_{D/L}$ (or $r_{L/D}$) = resolution coefficient = ratio of capacity factors (k_2:k_1) of the enantiomers; r_0 (solvent peak) = 1.5 min.

the mechanism of their stereoselective interaction with helicenes and (**1**), respectively, should be similar. The inversion of the order of emergence for the enantiomers of IX and X should be compared with the behaviour of the homologous phenylalanine derivatives (VII, VIII). It can be inferred that the intra- and inter-molecular interactions of the phenyl group are markedly different in the crowded α-position compared with the β-position.

Similarly, the non-resolvability of the Dnp-leucine methyl ester (III), as compared with the high *r* value found for the corresponding isoleucine derivative (IV), indicates the strong dependence of stereoselectivity on the position of the methyl substituent (γ- *vs.* β-) in the side chain of the amino-acid.

Higher *r* values are found, in general, for the isopropyl esters than for the methyl esters.

Retention times are strongly affected by the nature of both R^1 and R^2, as well as by eluant composition. For I—VI, the order of decreasing elution time is Ala > Val > Ile > Leu. Replacement of the methyl ester group by an isopropyl ester group considerably decreases retention (compare I with II).

By a judicious choice of derivatives, chiral donor reagent, and mobile phase composition, it should be possible to develop the present approach into a general tool for the enantiomeric analysis of Dnp-protein amino-acids.

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