Enantiomer separation of [60]fullerene derivatives by micro-column high-performance liquid chromatography using (R)-(−)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy) propionic acid as chiral stationary phase

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Abstract

The enantiomer separation of [60]fullerene derivatives on the chiral stationary phase (CSP) (R)-(−)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy) propionic acid (TAPA) was achieved. A [60]fullerene tris- and hexakis-adduct with achiral addends and an inherent chiral addition pattern were separated on a micro HPLC column containing R-(−)-TAPA bonded to silica gel as CSP. The stationary phase exhibits an intermediate polarity and separations were performed using the normal and reversed-phase mode. With the hexakis-adduct, near-baseline separation was achieved. The tris-adduct shows a strong interaction with the CSP expressed by a high retention factor but inferior enantiomer separation.   © 1997 Elsevier Science B.V.

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1. Introduction

The application of 2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy) propionic acid (TAPA) as chiral stationary phase (CSP) in high-performance liquid chromatography (HPLC) was first reported by Gil-Av and co-workers. [1,2] for the successful enantiomer separation of several racemic carbohelicenes. Since then, TAPA coated columns have been used in charge-transfer chromatography for the separation of numerous chiral aromatic compounds [3–6]. TAPA, a strong electron accepting agent due to the tetranitrofluorenylidene moiety, can form charge-transfer complexes with appropriate electron donating solutes. π-Donor–acceptor interactions and one stereochemically significant interaction via hydrogen bonds are required for chiral discrimination [7].

Charge-transfer chromatography is also well suited for the separation of fullerenes and fullerene derivatives with respect to the fullerene based π-system. Guiochon and co-workers [8] reported the separation of C_{60} and C_{70} on TAPA bonded silica gel with a high separation factor. Investigations on chiral fullerene derivatives and their enantiomer separation by means of chromatography on a chiral stationary phase is a topic of increasing interest [9–12]. In fullerene chemistry there are several possibilities for

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obtaining chiral fullerene derivatives [13]. If the addends themselves are achiral, chirality arises from the nature of the addition pattern. Hirsch and co-workers [14–16] have reported the synthesis and characterization of several [60]fullerene adducts. Recently we reported the enantiomer separation of some of these inherently chiral fullerene derivatives on the well-known π-donor–π-acceptor CSP Whelk-O1 [17].

In this report we demonstrate the enantiomer separation of two [60]fullerene derivatives with an inherent chiral addition pattern by means of micro-column HPLC. For the first time chiral fullerene derivatives were separated on the chiral charge-transfer complexing agent TAPA bound to silica gel.

Micro-column HPLC [18–22] has proved to be a very useful method, when only limited amounts of the stationary phase are available and expensive and/or toxic solvents are used. Separation of a [60]fullerene tris- and hexakis-adduct was performed in the normal and the reversed-phase mode. Via circular dichroism (CD) spectra of the single en-antiomers, previously obtained on the analytical Whelk-O1 column [17], the elution order on R-(−)-TAPA bonded to silica gel was determined.

2. Experimental

2.1. Instrumentation

The micro-column HPLC system consisted of a Merck–Hitachi LaChrom pump L-7100 (Merck, Darmstadt, Germany) and a Linear UVIS-205 detector (Dionex, Idstein, Germany) equipped with a fused-silica capillary bubble-type detector flow cell (tubing 100 μm I.D., bubble 250 μm I.D., Grom Analytik, Herrenberg, Germany). The flow of the pump was split before injection. Injections were performed using a 60 nl internal loop Valco injection valve (Klaus Ziemer, Mannheim, Germany). The fused-silica capillary was end-connected to a Valco fitting containing stainless steel sieves (2 μm pores).

CD spectra were obtained on a J720 spectrometer from Asco (Tokyo, Japan) using a 0.02 cm path-length quartz cell with a resolution of 0.5 nm. CD spectra were recorded in acetonitrile at room temperature.

2.2. Column preparation

A 250 mm×0.25 mm I.D. fused-silica capillary was packed with the support at 300 bar using the slurry packing method. The slurry and packing medium was methanol. After the packing process the column was rinsed with a few ml CH_2Cl_2 containing 1,1,1,3,3,3-hexamethyldisilazane (Fluka, Buchs, Switzerland) as endcapping agent. The chiral station-ary phase was a gift from the late Professor Gil-Av (Weizmann Institute of Science, Rehovot, Israel), synthesized according to Kim et al. [5]. R-(−)-TAPA was chemically bonded to Lichrosorb Si 100 (5 μm, Merck). The binding of the organic material to silica was verified by elemental analysis (carbon content 8.51%).

2.3. Eluent and samples

Acetonitrile (LiChrosolv, gradient grade) was purchased from Merck. Toluene and n-heptane (HPLC grade) were purchased from Aldrich (Steinheim, Germany). Water was purified using a Millipore Q system. All different solvents were used without further purification.

The fullerene derivatives were prepared in the laboratory of Professor Hirsch (Erlangen, Germany).

2.4. Chromatographic measurements

The eluent flow-rate was in the range of 0.4–2.8 μl/min in all experiments. All measurements were performed at room temperature under isocratic con-ditions. The column void times t_0 were determined using toluene–n-heptane mixtures by injecting a small plug of pure toluene, in acetonitrile–water mixtures by the use of acetonitrile and recording the perturbation signal. Under acetonitrile–water con-ditions the hexakis-adduct was detected at 254 nm. The tris-adduct was detected at 315 nm in toluene–n-heptane mobile phase mixtures.

3. Results and discussion

In this report we describe the enantiomer sepa-ration of two chiral [60]fullerene derivatives on the CSP TAPA (Fig. 1). Scheme 1 shows the enantio-
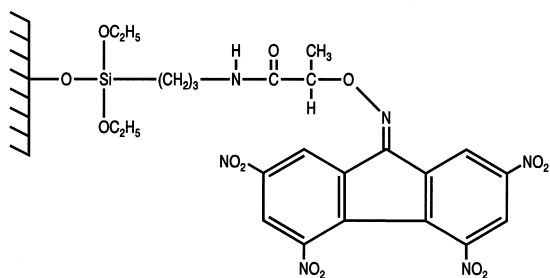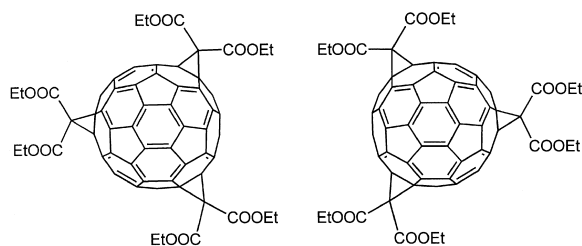
Fig. 1. 2-(2,4,5,7-Tetranitro-9-fluorenylideneaminooxy) propionic acid (TAPA) bound to silica gel.

Scheme 1.

mers of a D_3-symmetric tris-adduct (^fC)-I,III*,III* (1a) and (^fA)-I,III*,III* (1b) with a *trans*-3, *trans*-3, *trans*-3 addition pattern and the chiral C_1-symmetrical hexakis-adduct $C_{66}(COOEt)_{12}$ (2) of unknown structure were successfully separated. The latter derivative is a side product in the synthesis of the corresponding T_h-symmetrical hexakis-adduct. Mobile phases of different compositions were tested, since both fullerene derivatives are well soluble in toluene and in acetonitrile. The hexakis-adduct was separated using acetonitrile–water mixtures as mobile phase. A mixture of acetonitrile–water (75:25,

v/v) produces near-baseline separation with a resolution $R_s = 1.25$ and a retention factor of the second eluted enantiomer $k_2 = 18.4$ (Fig. 2). Measurements were performed using flow-rates from 0.4 to 2.8 μl/min. Higher mobile phase velocities lead to a loss of resolution. Increasing the percentage of water from 15% to 30% (v/v) leads to only small changes in the α-values from $\alpha = 1.07$ to 1.09, respectively. A resolution $R_s = 0.84$ was obtained with acetonitrile–water (80:20) and increasing the water content in the mixture to 30% separates the enantiomers with $R_s = 1.25$. At percentages of more than 30% water in acetonitrile no further improvement of the separation could be observed, although the k_2 values increase considerably, for example for acetonitrile–water (70:30) $k_2 = 37.2$. Although the hexakis-adduct shows a stronger interaction with the CSP with increasing percentages of water, there is nearly no effect on the resolution.

For the tris-adduct even higher retention factors can be observed under these reversed-phase conditions but no enantiomer separation was achieved. Using acetonitrile–water mixtures (90:10) the hexakis-adduct shows a retention factor $k = 0.95$, whereas for the tris-adduct $k = 13.7$ under the same conditions. With acetonitrile–water strong peak tailing is observed for the tris-adduct. These results clearly indicate that strong π-donor–π-acceptor interactions, expressed by large retention factors, are not sufficient for enantiomer separation in charge-transfer chromatography. If there are no or only weak enantioselective interactions with the chiral center of the CSP, no enantiomer separation can be achieved. The tris-adduct with only three substituents on the
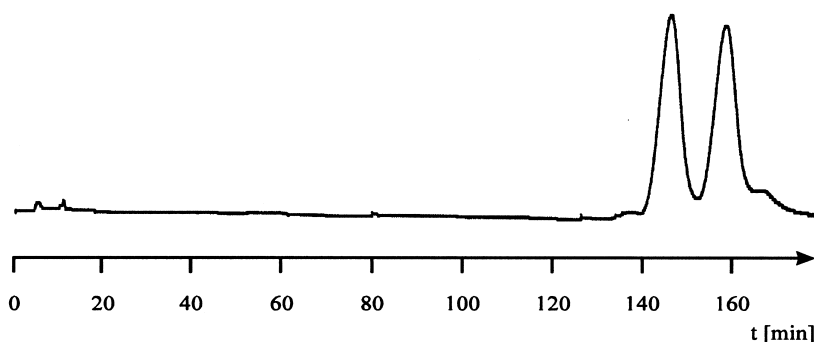

Fig. 2. Enantiomer separation of 2; conditions: acetonitrile–water (75:25, v/v); flow-rate 0.4 μl/min.

fullerene chromophore is sterically favoured for a multipoint interaction with TAPA. As for the investigated fullerene derivatives, chirality arises exclusively from the addition pattern at the carbon cluster itself, the chiral interaction is more effective with the hexakis-adduct, possessing more addends for the formation of hydrogen bonds.

Using the normal-phase mode with mixtures of toluene and *n*-heptane the D_3-symmetric tris-adduct can be separated into the enantiomers. The k_2 values range from $k_2 = 4.21$ for toluene–*n*-heptane (90:10) to $k_2 = 14.4$ for the mixture toluene–*n*-heptane (68:32). An increase of *n*-heptane in the mixture has almost no effect on the separation of the tris-adduct. The highest separation factor $\alpha = 1.06$ was achieved with toluene–*n*-heptane (80:20), increasing percentage of *n*-heptane to 32% leads to a negligible loss in the separation factor α ($\alpha = 1.05$) but to a slightly higher resolution (Fig. 3). A higher percentage of *n*-heptane in toluene leads to no further improvement in separation. Retention factors for the tris-adduct using toluene–*n*-heptane mixtures are lower than those obtained for the same compound in the reversed-phase mode. Even these extremely long retention times or high retention factors provide no baseline separation for the D_3-symmetric tris-adduct. Maybe this is due to the fact that charge-transfer agents require a high degree of planar recognition for chiral discrimination. This is a possible limitation for the interaction between R-(−)-TAPA and the shaped fullerene derivatives.

Surprisingly the C_3-symmetric tris-adduct with a *e,e,e*-addition pattern (3) could not be separated on R-(−)-TAPA using toluene–*n*-heptane mixtures as

mobile phase. Although both tris-adducts only differ in the substitution pattern of their addends, they show a remarkable different retention behaviour on TAPA. Whereas in toluene–*n*-heptane (80:20) 3 has a retention factor of $k = 4.10$, the second eluting enantiomer of 1 shows a value of $k_2 = 12.5$. The charge-transfer and in addition the chiral recognition ability is much weaker for 3. Steric hindrance and therefore weaker electron donating properties may explain these results.

The single enantiomers of 1 and 2 were isolated by semipreparative separation on the CSP Whelk-O1 [17]. The CD spectra are shown in Fig. 4 together with the corresponding elution order of the enantiomers on R-(−)-TAPA bound to silica gel.

4. Conclusions

Charge-transfer chromatography has proved to be a useful method for the separation of inherently chiral [60]fullerene derivatives. For the first time R-(−)-TAPA bound to silica gel was applied for fullerene enantiomer separation. The fullerene derivatives show high retention factors effected by strong π-donor–acceptor interactions with the CSP. The hexakis-adduct was nearly baseline separated showing a resolution of $R_s = 1.25$, whereas the tris-adduct despite of high retention factors shows only partial separation of the enantiomers. It can be seen that a strong charge-transfer interaction is a necessary but not a sufficient requirement for chiral discrimination. So far only Whelk-O1 columns have been employed for the enantiomer separation of
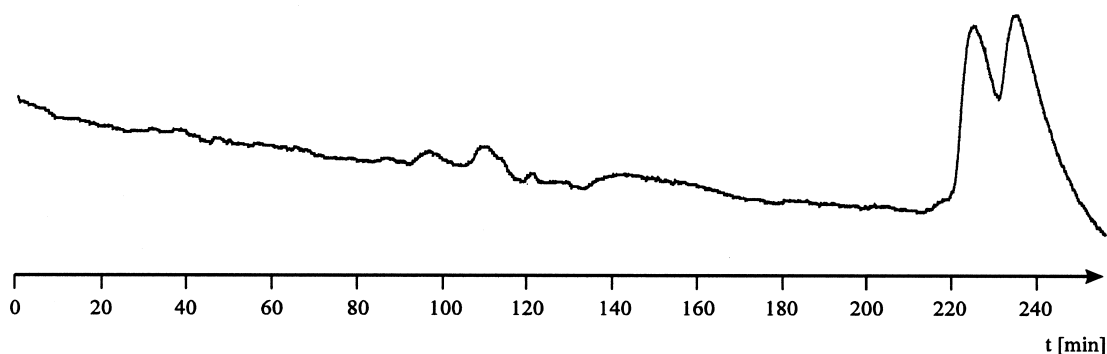

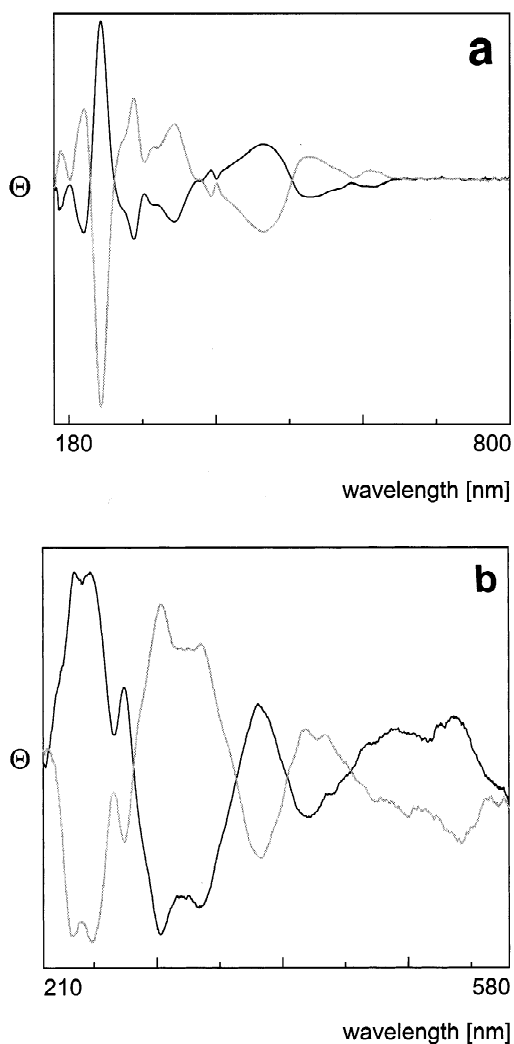Fig. 3. Enantiomer separation of 1; conditions: toluene–*n*-heptane (68:32, v/v); flow-rate 0.5 μl/min.

Fig. 4. Circular dichroism spectra of the enantiomers 1 and 2: (a) 1; (b) 2. grey curve: second eluted enantiomer; black curve: first eluted enantiomer.

chiral fullerene derivatives. TAPA bound to silica gel offers an additional important possibility for the separation of these compounds by means of HPLC.

References

[1] F. Mikeš, G. Boshart, E. Gil-Av, J. Chem. Soc., Chem. Commun. (1976) 99

[2] F. Mikeš, G. Boshart, E. Gil-Av, J. Chromatogr. 122 (1976) 205.

[3] H. Numan, R. Helder, H. Wynberg, Rec. Trav. Chim. 95 (1976) 211.

[4] B. Feringa, H. Wynberg, J. Am. Chem. Soc. 99 (1977) 602.

[5] Y.H. Kim, A. Tishbee, E. Gil-Av, J. Chem. Soc., Chem. Commun. (1981) 75

[6] M. Funk, H. Frank, F. Oesch, K.L. Platt, J. Chromatogr. A 659 (1994) 57.

[7] W.H. Pirkle, T.C. Pochapsky, Chem. Rev. 89 (1989) 347.

[8] M. Diack, R.N. Compton, G. Guiochon, J. Chromatogr. 639 (1993) 129.

[9] S.R. Wilson, Y. Wu, N.A. Kaprinidis, D.I. Schuster, C.J. Welch, J. Org. Chem. 58 (1993) 6548.

[10] A. Herrmann, F. Diederich, Helv. Chim. Acta 79 (1996) 1741.

[11] Y. Wang, D.I. Schuster, S.R. Wilson, C.J. Welch, J. Org. Chem. 61 (1996) 5198.

[12] J.-F. Nierengarten, V. Gramlich, F. Cardullo, F. Diederich, Angew. Chem., Int. Ed. Engl. 35 (1996) 2101.

[13] F. Diederich, C. Thilgen, A. Herrmann, Nachr. Chem. Technol. Lab. 44 (1996) 9.

[14] A. Hirsch, I. Lamparth, H.R. Karfunkel, Angew. Chem., Int. Ed. Engl. 33 (1994) 437.

[15] F. Djojo, A. Herzog, I. Lamparth, F. Hampel, A. Hirsch, Chem. Eur. J. 2 (1996) 1537.

[16] I. Lamparth, C. Maichle-Mössmer, A. Hirsch, Angew. Chem., Int. Ed. Engl. 34 (1995) 1607.

[17] B. Gross, V. Schurig, I. Lamparth, A. Herzog, F. Djojo and A. Hirsch, J. Chem. Soc., Chem. Commun., (1997) 1117.

[18] R.P.W. Scott, J. Chromatogr. 125 (1976) 251.

[19] D. Ishii, K. Asai, K. Hibi, T. Jonokuchi, M. Nagaya, J. Chromatogr. 144 (1977) 157.

[20] R.P.W. Scott, Adv. Chromatogr. 22 (1983) 247.

[21] M. Nowotny, Anal. Chem. 60 (1988) 500A.

[22] M. Goto, T. Takeuchi, D. Ishii, Adv. Chromatogr. 30 (1989) 167.