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Direct resolution of C_{76} enantiomers by HPLC using an amylose-based chiral stationary phase

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A chiral fullerene C_{76} was directly resolved by high-performance liquid chromatography (HPLC) using a chiral stationary phase, amylose tris(3,5-dimethylphenylcarbamate), bonded onto silica gel and the mirror-imaged CD spectra of the enantiomers were in good pattern agreement with those of the previous report.

In 1993, Hawkins and Meyer reported the kinetic resolution of C_{76} enantiomers by asymmetric osmylation,[1] and this method was successfully applied to the resolution of other chiral fullerenes, C_{78} and C_{84}.[2] However, direct resolution of chiral fullerenes by HPLC with a chiral column appears very difficult judging from the tiny structural differences in the C_{76} enantiomers as pointed out by Hawkins and has so far been unsuccessful,[1] although chiral fullerene C_{60} derivatives with achiral substituents have been successfully resolved into enantiomers using chiral stationary phases (CSPs) based on a cellulose derivative[3] and a tetranitrofluorenylidene derivative.[4]

Phenylcarbamate derivatives of polysaccharides, particularly cellulose and amylose, show high chiral recognition abilities when used as CSPs for HPLC.[5–7] Among the many derivatives, 3,5-dimethylphenylcarbamates of cellulose[8] and amylose[9] are very useful CSPs with an excellent ability of resolving a wide range of racemates. These CSPs have been prepared by coating or adsorbing the polysaccharide derivatives on silica gel, and therefore cannot be used with solvents such as $CHCl_3$ and THF that dissolve or swell the derivatives. On the other hand, amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) bonded to silica gel only at its terminal residue has been prepared *via* enzymatic polymerization, and can be used with $CHCl_3$ and THF.[10]

In the present study, we succeeded in the first direct HPLC resolution of the smallest chiral fullerene C_{76} on the chemically bonded Chiralpak AD using a hexane–$CHCl_3$ (80:20) mixture as the eluent.

The racemic C_{76} sample was isolated as a chlorobenzene solution by HPLC and identified by mass spectroscopy. The concentration of the solution was estimated to be *ca.* 0.36 mM l^{-1} based on the UV spectrum measurement.[11] The chemically bonded Chiralpak AD column was prepared by our group in Nagoya.[10] Chromatographic resolution was performed on a modified JASCO PU-980 chromatograph equipped with a UV and CD dual detector (JASCO CD-1595) and a recycle valve unit (HV-1592-01). The racemic C_{76} solution (100 μl) was injected into the chromatographic system with a Rheodyne Model 7125 injector. A hexane–$CHCl_3$ (80:20) mixture was used as the eluent at a flow rate 1.0 ml min^{-1}. UV spectra were measured in hexane–$CHCl_3$ and toluene solutions using a JASCO Ubest V-570 spectrophotometer. The CD spectra of the C_{76} enantiomers in hexane–$CHCl_3$ and toluene were taken on a JASCO J-720 L apparatus with a 0.2 cm quartz cell at ambient temperature.

When the resolution of the racemic C_{76} sample was examined on the commercial Chiralcel OD and Chiralpak AD columns using a hexane–propan-2-ol mixture as the eluent, the elution of the sample was not clearly observed probably because of very low solubility of C_{76} in this eluent which resulted in a very long retention time. This forced us to use chiral columns that can be used with a $CHCl_3$ eluent system. We then examined the resolution on the chemically bonded Chiralpak AD and Chiralcel OD phases using the hexane–$CHCl_3$ eluent, and found that Chiralpak AD had better resolution. The results of the resolution are shown in Fig. 1. Both the UV and CD signals were monitored by the dual detector. The UV signal always showed a single peak sometimes with a shoulder, while the CD signal monitored at 400 nm showed the first (+) peak followed by the second (–) peak. This indicates that a low degree of resolution was attained and in the first cycle two (+) peaks were clearly observed. The reason for the appearance of the two peaks is not immediately clear, but may be due to sample overloading. Very low loading capacity of the column seems to indicate that chiral recognition of the C_{76} enantiomers may be performed at specific chiral sites containing multi amylose chains. In all the recycle separations, only the front main part of the (+) fraction was recycled, and the late main portion of the (–) fraction was collected during each recycle. Therefore, as the number of recycles increased the UV intensity decreased, and after 8 recycles, almost no (–) peak was observed. After 5 recycles, the intensity ratio of the UV and CD detection at the peak top became almost constant. These results suggest that the (+) isomer after 8 recycles may have a higher enantiomeric excess (ee).

The HPLC analysis of the (+) and (–) fractions resolved by the recycling are shown in Fig. 2. The (+) fraction shows a very good agreement between the UV and CD curves indicating that this fraction has a high enantiomeric purity. On the other hand, the (–) fraction shows a small (+) peak besides a main (–) peak, indicating lower enantiomeric purity of this fraction.

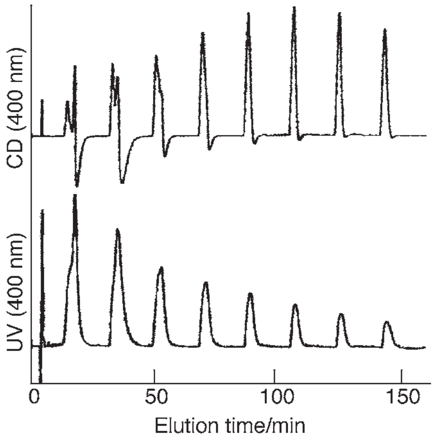

Fig. 1 Chromatographic recycled resolution of C_{76} on chemically bonded Chiralpak AD column. Column, 25 × 0.46 cm (id); eluent, hexane–$CHCl_3$ (80:20); flow rate, 1.0 ml min^{-1}.

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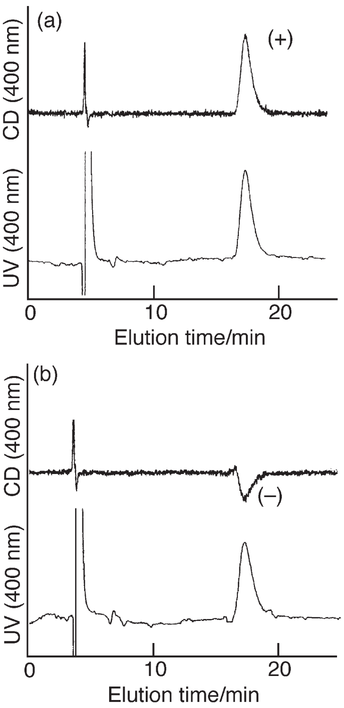

Fig. 2 HPLC chromatograms of the first (a) and the second (b) eluted enantiomers isolated after recycled resolution of C_{76}. (Column, chemically bonded Chiralpak AD; eluent, hexane–$CHCl_3$ (80:20); flow rate, 1.0 ml min^{-1}.)

Fig. 3 shows the CD spectra of the obtained C_{76} enantiomers in hexane–$CHCl_3$. When the solvent was replaced with toluene, almost identical spectra were observed. The spectral pattern is very similar to that for the C_{76} isolated by kinetic resolution,[1] but the intensity for the (+) fraction in this study is about 1.6 times greater than that for the above C_{76} which has been expected to have nearly 100% enantiomeric purity.[1] This means that our C_{76} would impossibly have over 100% ee based on the reported data. Since we used the same UV data[11] to estimate the C_{76} concentration,[12] the sample isolated in ref. 1 might have a lower enantiomeric purity, or the UV and CD measurements in ref. 1 might contain some error. Therefore, the reason for this disagreement in the CD data is not clear at this stage.

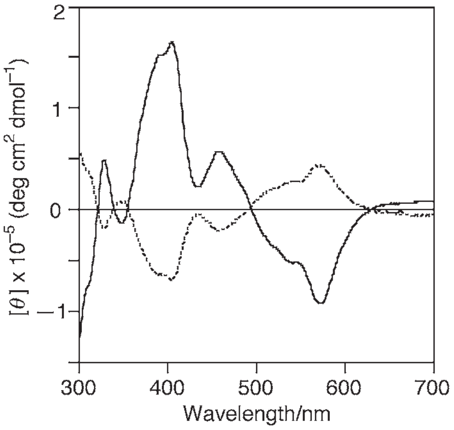

Fig. 3 CD spectra of C_{76} (first eluted enantiomer, solid line; second eluted enantiomer, dotted line) in toluene.

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12 The concentration of the isolated samples was estimated in a 2.0 mm cell based on $\varepsilon = 28150$ at 330 nm in ref. 11. The concentration of the first eluted enantiomer of C_{76} was 7.76×10^{-5} M and the CD intensity at 330 nm was 7.5 mdegree.