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# Chiral resolution of enantiomers of asymmetric cobaltacarboranes with a monoatomic bridge between ligands by liquid chromatography on a β-cyclodextrin column

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ABSTRACT

The HPLC resolution on a β-cyclodextrin column is reported of thirteen enantiomeric pairs of racemates of the type 6,6′-μ-R-E(1,7-$C_2B_9H_{10})_2$-2-Co with oxygen, sulphur and nitrogen bridges (E) and with a variety of R substituents differing in size and electronic properties along with structural factors influencing the capacity factors, selectivity and resolution of individual compounds. A semi-preparative method for chiral separations of these racemates is described and circular dichroism (CD) spectra of several pure enantiomers are presented. The CD spectra indicate the same absolute configurations of all enantiomers eluted first or last independent of the nature of the bridging atom and the size of the bridge substituting group R.

## INTRODUCTION

Covalently bonded β-cyclodextrin chiral stationary phases (CSPs) have been widely used for the resolution of enantiomers of organic chiral compounds [1–5]. The separation of several metallocene derivatives on this type of CSP has also been described [6]. Both studies of the intercalation complexes of metallocenes with cyclodextrins [7–9] and the investigation of the separation mechanism of selected enantiomers on β-cyclodextrin columns [10–12] suggest that the inclusion process of the solute with the cyclodextrin hydrophobic cavity along with additional interactions (hydrogen bonding, steric, etc.) with hydroxyl groups on the rim of the cyclodextrin cavity are responsible for the enantiomer discrimination.

Recent studies in this laboratory have shown the efficiency of covalently linked β-cyclodextrin CSPs for the HPLC resolution of the first eleven enantiomeric pairs of chiral deltahedral carborane derivatives [13,14]. In this investigation, of four protochiral metallaborane derivatives tested, only one pair of enantiomers was successfully resolved.

Another structural type of metallacarborane complex suitable for HPLC resolution of enantiomers on β-cyclodextrin columns [15] is the monoatomic bridge cobaltacarboranes of the type 6,6′-μ-R-E(1,7-$C_2B_9H_{10})_2$-2-Co (Fig. 1). The constitutions of the first two representatives of this series have been determined by multinuclear NMR

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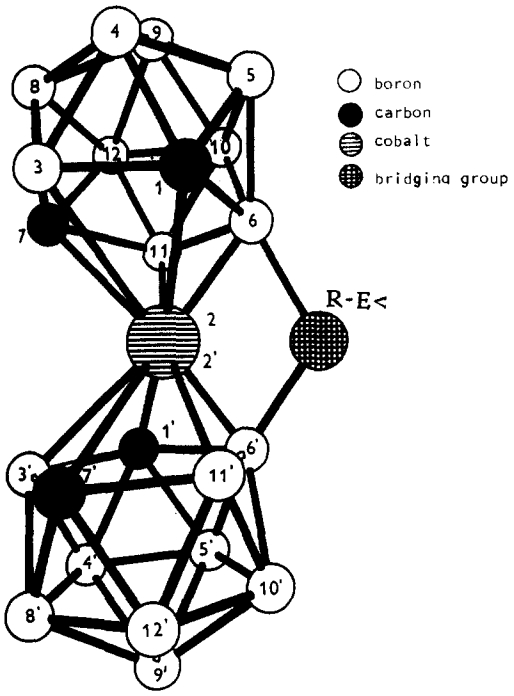


Fig. 1. Structure of the asymmetric metallaboranes of the 6,6′-μ-R-E(1,7-$C_2B_9H_{10}$)$_2$-2-Co (E = O, S, N) type. Terminal hydrogens are omitted for clarity.

spectroscopy and confirmed by resolution of enantiomers [16].

Both of the above-mentioned sulphur-bridged species are just examples of a large family of potentially available compounds of this type because of the possible variation of the bridge substituent and the bridging atom itself. The main structural features will remain essentially the same for all members of this family, *e.g.*, the rigid helical arrangement of both ligands with consequent protochirality without any individual chiral centre joined to a single atom. Moreover, the charge non-equivalence of C–H and B–H vertices at the circumference of both pentagonal ligand planes renders the molecule strongly quadrupolar in the area adjacent to the central cobalt atom. The known hydrophobicity of large deltahedral *closo* frameworks is another interesting and general feature of such species.

Such features are entirely different from conventional organic compounds. The chiral resolution of these unique cobaltacarborane compounds might reveal some additional factors essential for chiral resolution on β-cyclodextrin CSPs.

## EXPERIMENTAL

### *Column*

A stainless-steel (250 × 8 mm I.D.) HPLC column was slurry packed with β-cyclodextrin CSP having a high cyclodextrin loading (carbon content 9%). Synthesis of the CSP with β-cyclodextrin directly bonded on the spherical silica support (Separon SGX, 7 μm; Tessek, Prague, Czechoslovakia) was made according to the previously described procedure [15]. The void volume of the β-cyclodextrin column was determined according to the literature method [2].

### *Apparatus*

The chromatographic equipment consisted of a pulseless dual-piston high-pressure VCR 40 pump (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia), a Rheodyne (Cotati, CA, USA) Model 7125 sampling valve with 10- or 200-μl loops, an LCD 2040 variable-wavelength (190–560 nm) UV spectrophotometric detector (Laboratory Instruments, Prague, Czechoslovakia), a Servogor 2s line recorder (Brown Boveri, Germany), and a CI 100 integrator (Laboratory Instruments).

### *Chemicals, sample preparation and detection*

Deionized water was used for the preparation of aqueous–organic mobile phases. All other chemicals were of analytical-reagent grade (Lachema Brno, Czechoslovakia). Methanol was distilled before use. Eluents were filtered through a 0.45 μm filter and briefly degassed under vacuum.

All deltahedral borane compounds (**1–13**, Tables I and II) were prepared in the Boron Chemistry Group of the Institute of Inorganic Chemistry, Czechoslovak Academy of Sciences. The syntheses and properties of the still not reported zwitterionic compounds 6,6′-μ-R-S(1,7-$C_2B_9H_{10}$)$_2$-2-Co (**2–8**; R = ethyl, *n*-propyl, isopropyl, allyl, *n*-butyl, methoxycarbonylmethyl, *n*-hexyl), 6,6′-μ-$R_1$,$R_2$-N(1,7-$C_2B_9H_{10}$)$_2$-2-Co (**11**, $R_1$ = $R_2$ = H; **12**, $R_1$ = methyl, $R_2$ = H; **13**, $R_1$ = $R_2$ = methyl) and 6,6′-μ-Me-O(1,7-$C_2B_9H_{10}$)$_2$-2-Co (**10**) will be the subject of a separate paper [17]. The synthesis and

properties of the 6,6′-$\mu$-Me-S(1,7- $C_2B_9H_{10})_2$-2-Co (**1**) and its parent $S^{(-)}$ < (**9**) bridged anion have been reported recently [16].

Special attention was paid to ensure the purity of the individual protochiral positional isomers used in this study. The purity of all species was checked by $^1H$ (at 500 MHz) and $^{11}B$ (at 160 MHz) NMR and mass spectrometry.

Samples were prepared as methanolic solutions of concentration 1.0 mg/ml. Before injection all samples were filtered through a 0.45-$\mu$m PTFE microfilter (Tessek).

The sulphur and oxygen bridge compounds (**1–10**, Tables I and II) were detected at 290 nm and the analogous nitrogen bridge compounds (**11–13**, Table II) at 280 nm.

*Circular dichroism spectra*

The solutions of enantiomers (see *Semipreparative separations*) accumulated from twelve successive injections of 40 $\mu$l of the solution of the given racemate (concentration 1.0 mg/ml) were evaporated to dryness under vacuum, the residue was dissolved in 4 ml of methanol and the resulting solutions were used directly for circular dichroism (CD) spectral measurements.

CD spectra were recorded on an Auto Dichrographe MarkV instrument (Jobin Yvon, France). The instrument is driven by a microcomputer (Silex, France) loaded with our own software. The measurements were performed in quartz cells with an optical path length of 1 or 0.1 cm. The spectra are computer averages over 2–3 instrument scans and the intensities are presented in arbitrary units.

## RESULTS AND DISCUSSION

The zwitterionic S-bridge compounds used in this study are summarized in Table I together with their retention data in 85% and 78% aqueous methanol, selectivity ($\alpha$) and resolution ($R_s$). The $k'$ and $R_s$ values of these compounds indicate the usual retention behaviour observed on $\beta$-cyclodextrin CSPs by varying the organic modifier content in the mobile phase (studied in the range 70–90%) [1,2,14]. The high methanol content necessary for elution gave evidence of a strong hydrophobic interaction of these compounds with $\beta$-cyclodextrin CSPs. However, the more hydrophilic (because of its charge) parent anion [6,6′-$\mu$-S(1,7-$C_2B_9H_{10})_2$-2-Co]$^-$ (**9**, Table II) could be resolved only in mobile phases with lower methanol concentrations (50–60%). In the series of zwitterrionic substituted sulphur compounds (**1–8**, Table I), the effect of increasing the substituent size is generally to decrease the capacity factors ($k'$). Maximum resolution was obtained with the ethyl-substituted compound, which was followed by a decreasing trend as the size of the

TABLE I

CAPACITY FACTORS ($k'$), SELECTIVITY ($\alpha$) AND RESOLUTION ($R_s$) OF ENANTIOMERS OF COMPOUNDS OF THE 6,6′-$\mu$-R-S(1,7-$C_2B_9H_{10})_2$-2-Co TYPE ON A DIRECTLY BONDED $\beta$-CYCLODEXTRIN (7 $\mu$m) COLUMN (250 × 8 mm I.D.) USING AQUEOUS–METHANOLIC MOBILE PHASES

| R | No. | 85% Methanol[a] | | | 78% Methanol[b] | | |
|---|---|---|---|---|---|---|---|
| | | $k'_1$[c] | $\alpha$ | $R_s$ | $k'_1$[c] | $\alpha$ | $R_s$ |
| Me | **1** | 7.50 | 1.17 | 1.08 | 16.11 | 1.19 | 1.05 |
| Et | **2** | 5.92 | 1.20 | 1.87 | 12.0 | 1.31 | 2.06 |
| *n*-Pr | **3** | 4.42 | 1.12 | 1.41 | 7.89 | 1.16 | 1.47 |
| *i*-Pr | **4** | 4.21 | 1.11 | 1.33 | 7.06 | 1.17 | 1.50 |
| $CH_2$=CH-$CH_2$ | **5** | 4.67 | 1.12 | 1.27 | 7.72 | 1.15 | 1.42 |
| *n*-Bu | **6** | 3.58 | 1.10 | 1.05 | 6.50 | 1.09 | 1.13 |
| MeOCO$CH_2$ | **7** | 4.25 | 1.10 | 1.0 | 7.67 | 1.13 | 1.22 |
| *n*-Hex | **8** | 3.0 | 1.09 | 0.94 | 5.85 | 1.07 | 0.7 |

[a] Flow-rate 1.2 ml/min.

[b] Flow-rate 1.6 ml/min.

[c] $k'_1$ = Capacity factor of the first-eluting enantiomer.

substituent increased. However, for substituents up to *n*-butyl or methylacetyl, relatively good resolutions ($R_s > 1$) were still obtained.

The separation is exemplified by Fig. 2. The poor $R_s$ value for the methyl derivative **1** was not the result of selectivity, which was almost as good as that of the ethyl derivative, but rather to the peak broadening. Therefore, the methyl derivative **1** seems to follow to some extent the behaviour of the parent anion with a relatively high $\alpha$ value and extensively broadened peaks. Comparison of the chromatographic behaviour of series of compounds with a three-carbon chain as the substituent group (**3–5**) revealed only slight differences in capacity factors and resolution values. Also, no substantial enhancement of resolution was observed for **7** with a more hydrophilic methylacetate substituent. The correlation of decreasing capacity factors with increasing size of the substituent groups would be consistent with increasing disruption of the intercalation process between the hydrophobic carborane part of the compounds and the cyclodextrin cavity as the size of the side substituents bonded to the rigid molecule increases.

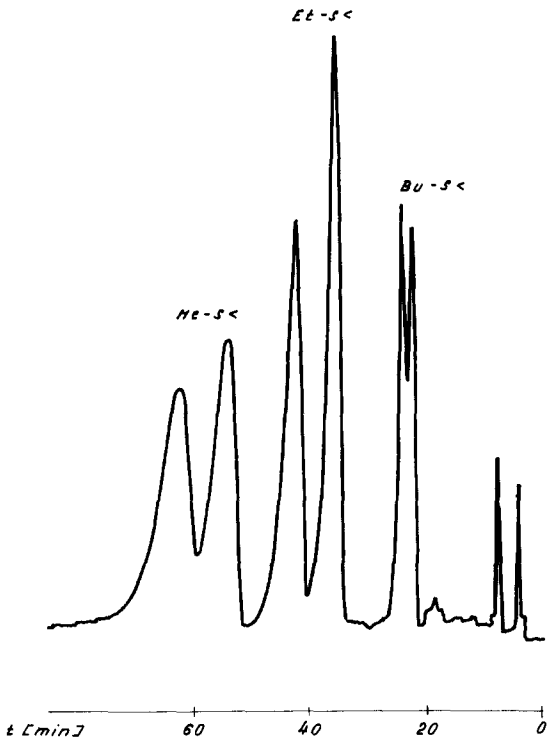


Fig. 2. Separation of the enantiomers of the zwitterionic species of the 6,6′-$\mu$-R-S(1,7-$C_2B_9H_{10}$)$_2$-2-Co type with BuS<, EtS< and MeS< bridges. Column, $\beta$-cyclodextrin (250 × 8 mm I.D.); mobile phase, 80% aqueous methanol; flow-rate 1.2 ml/min; detection, UV at 290 nm; sensitivity, 0.04 a.u.f.s.

TABLE II

CAPACITY FACTORS ($k'$), SELECTIVITY ($\alpha$) AND RESOLUTION ($R_s$) OF ENANTIOMERS OF COMPOUNDS OF THE 6,6′-$\mu$-R-E(1,7-$C_2B_9H_{10}$)$_2$-2-Co (E = O,S) AND 6,6′-$\mu$-R$_2$-N(1,7-$C_2B_9H_{10}$)$_2$-2-Co TYPE ON A DIRECTLY BONDED $\beta$-CYCLODEXTRIN (7 $\mu$m) COLUMN (250 × 8 mm I.D.) IN AQUEOUS–METHANOLIC MOBILE PHASES

Flow-rate, 1.6/min.

| Bridge | No. | $k'_1$[a] | $\alpha$ | $R_s$ | MeOH (%) |
|---|---|---|---|---|---|
| S(−)< | **9** | 8.0 | 1.37 | 0.85 | 55 |
| MeO<[b] | **10** | 4.0 | 1.72 | 1.30 | 56 |
| $H_2N$< | **11** | 7.33 | 1.12 | 0.95 | 75 |
| MeHN< | **12** | 7.83 | 1.16 | 1.06 | 78 |
| $Me_2N$< | **13** | 7.67 | 1.23 | 1.47 | 85 |

[a] $k'_1$ = Capacity factor of the first-eluting enantiomer.
[b] Demethylation occurs, enantiomers are eluted as [6,6′-$\mu$-O< (1,7-$C_2B_9H_{10}$)$_2$-2-Co]$^-$ anions.

Additionally, three compounds with nitrogen (**11–13**) and one with oxygen (**10**) monoatomic bridges can also be resolved on this type of CSP. Table II summarizes their retention data, selectivity and resolution achieved using the optimum mobile phase composition for each compound. The separation of the enantiomers of three zwitterionic nitrogen bridge compounds, the parent [6,6′-$\mu$-$H_2N$ (1,7-$C_2B_9H_{10}$)$_2$-2-Co (**11**) and its monomethyl and dimethyl derivatives (**12** and **13**), is depicted in Fig. 3. The retention and selectivity trends in this series of nitrogen-bridged compounds can be clearly seen from Table II and Fig. 3. The strength of interaction with $\beta$-cyclodextrin increases with the degree of nitrogen alkylation similarly to the selectivity and resolution. The $R_s$ values for the $Me_2N$<-bridged compound **13** were generally better than those for the corresponding methylated sulphur-bridged compound **1**. The worst $\alpha$ and $R_s$ values were obtained for the parent $H_2N$< compound (**11**) with acidic hydrogens on the nitrogen bridge. The compound with an MeO< bridge (**10**) exhibited surprisingly low hydrophobicity, as can be seen from

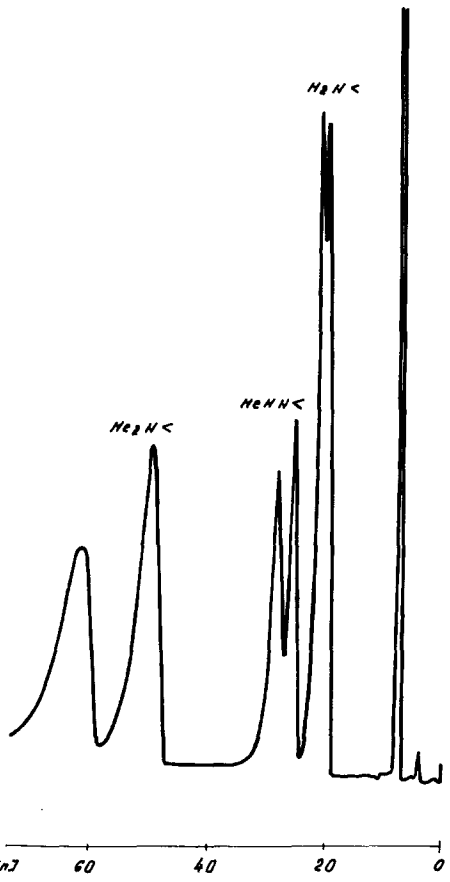


Fig. 3. Separation of the enantiomers of the zwitterionic $H_2N<$ (**11**), MeHN< (**12**) and $Me_2N<$ (**13**) bridged compounds of the 6,6′-$\mu$-$R_2$-N(1,7-$C_2B_9H_{10})_2$-2-Co type. Chromatographic conditions as in Fig. 2, except for detection at 280 nm.

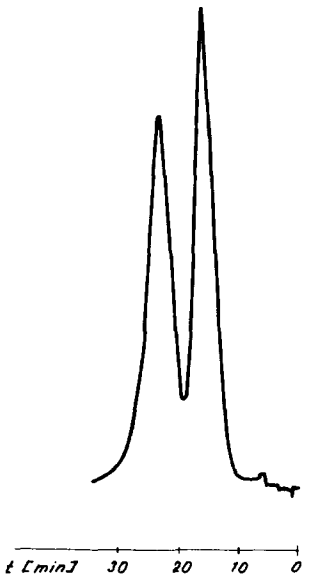


Fig. 4. Separation of the enantiomers of 6,6′-$\mu$-Me-O(1,7-$C_2B_9H_{10})_2$-2-Co (**10**). Conditions as in Fig. 2, except for methanol concentration 60%.

the low organic modifier content used for its elution (Table II). Additionally, it was proved that the demethylation to the anion with a bare oxygen bridge took place during the chromatographic process and the enantiomers were eluted as the respective conjugate acids. An example of such a separation is given in Fig. 4. As with the sulphur-bridged anion (**9**), compound **10** gives a high selectivity value, but the decrease in resolution is apparent by extensive band broadening.

It is noteworthy that no bridge substituent is needed for a successful resolution of this class of compounds, as shown by examples of the separation of parent compounds with $S^{(-)}<$, $O^{(-)}<$ and $H_2N<$ bridges (**9, 10** and **11**). In these species, similarly as in **13**, the primary coordination sphere around the bridging atom is not chiral; however, the whole molecules are helically twisted (Fig. 1). Therefore, for the successful resolution of enantiomers of **1–13** on $\beta$-cyclodextrin columns, only general asymmetry of these molecules is essential. Therefore, no distinct monoatomic chiral centres need be present.

*Semi-preparative separations*

The successful resolution of species **1–7, 10, 12** and **13** with resolution values better than 1 led us to develop a semi-preparative procedure for the accumulation of pure enantiomers in amounts sufficient for the measurement of their circular dichroism spectra and for X-ray diffraction studies aimed at determinating absolute configurations. Therefore, a study of the dependence of the capacity factors and resolution values on the sample loading was performed on the 250 × 8 mm I.D. column used throughout this work.

The results for the allyl-S<-bridged compound (**5**) for two mobile phase compositions are shown in Table III. In the mobile phase with a lower metha-

TABLE III

EFFECT OF SAMPLE LOADING ON THE CAPACITY FACTORS ($k'$) AND THE RESOLUTION ($R_s$) OF THE ENANTIOMERS OF 6,6'-$\mu$-ALLYL-S(1,7-$C_2B_9H_{10})_2$-2-Co (**5**)

Column, $\beta$-cyclodextrin (7 $\mu$m) (250 × 8 mm I.D.); mobile phase, A = 77% and B = 83% aqueous methanol; flow-rate, 1.6 ml/min; detection, UV at 290 nm; injections, 1–200 $\mu$l of methanolic solution of concentration 1 mg/ml.

| Injection volume ($\mu$l) | Mobile phase A | | | | Mobile phase B | | | |
|---|---|---|---|---|---|---|---|---|
| | $k'_1$ | $k'_2$ | $\alpha$ | $R_s$ | $k'_1$ | $k'_2$ | $\alpha$ | $R_s$ |
| 1 | 10.10 | 11.55 | 1.15 | 1.45 | 5.25 | 5.81 | 1.11 | 1.31 |
| 5 | 10.0 | 11.33 | 1.13 | 1.30 | 5.25 | 5.75 | 1.10 | 1.28 |
| 10 | 9.33 | 10.63 | 1.14 | 1.17 | 5.25 | 5.81 | 1.11 | 1.25 |
| 20 | 9.11 | 10.32 | 1.13 | 1.10 | 5.25 | 5.75 | 1.10 | 1.25 |
| 40 | 8.55 | 9.66 | 1.13 | 1.05 | 5.25 | 5.75 | 1.10 | 1.20 |
| 50 | 8.22 | 9.33 | 1.14 | 0.96 | 5.25 | 5.75 | 1.10 | 1.20 |
| 80 | 8.0 | 8.88 | 1.11 | 0.77 | 5.13 | 5.68 | 1.11 | 1.06 |
| 100 | 7.91 | 8.62 | 1.09 | 0.65 | 5.13 | 5.68 | 1.11 | 0.87 |
| 200 | – | – | – | – | 5.06 | 5.45 | 1.08 | 0.72 |

nol content (77%), it is apparent that both $k'$ and $R_s$ have decreased relatively rapidly with increasing loading of the sample (from 1.0 to 100 $\mu$g). In contrast, with a mobile phase with a methanol content of 83%, the $k'$ values have been less affected and the $R_s$ values decreased less sharply with increasing sample loading. Therefore, it seems that the observed decreases in $R_s$ can be attributed to kinetic factors (poor mass transport in mobile phases with limited solubility of solutes) rather than to a true overloading of the stationary phase. From these results it follows that the mobile phases with higher methanol contents are to be preferred for preparative applications, thus permitting the separation of a large amount of sample with a single injection (*ca.* 80 $\mu$g).

Collection of the eluent from the start up to the upper quarter of the descending part of the first peak afforded the faster moving enantiomer in nearly 100% purity. The fraction collected from the

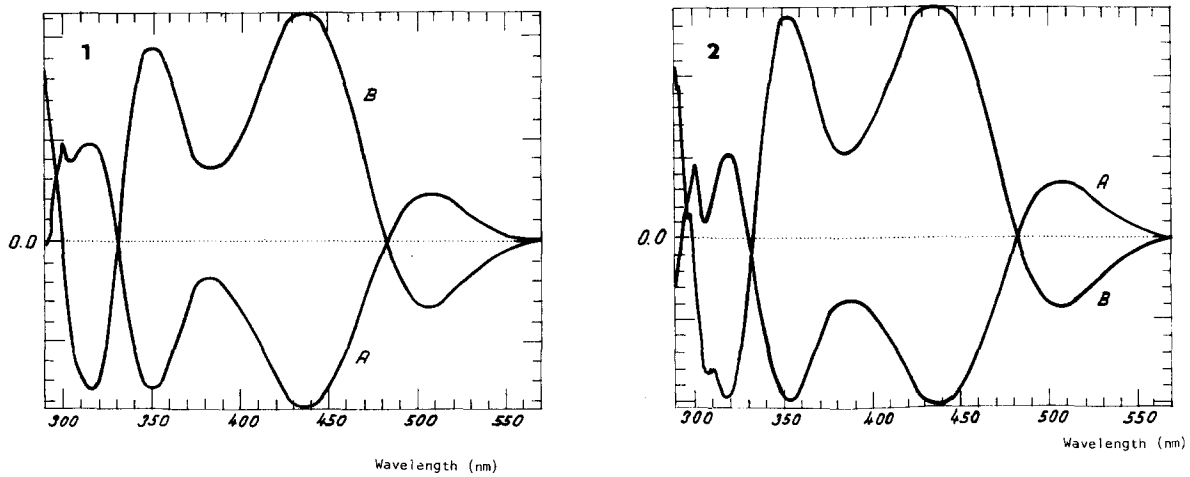


Fig. 5. CD spectra (wavelength range 300–580 nm) of the enantiomers of compounds **5** and **7**. (1) 6,6'-$\mu$-allyl-S(1,7-$C_2B_9H_{10})_2$-2-Co enantiomers; (2) 6,6'-$\mu$-MeOCOCH$_2$-S(1,7-$C_2B_9H_{10})_2$-2-Co enantiomers. Curves A and B represent the first- and second-eluting enantiomers, respectively.

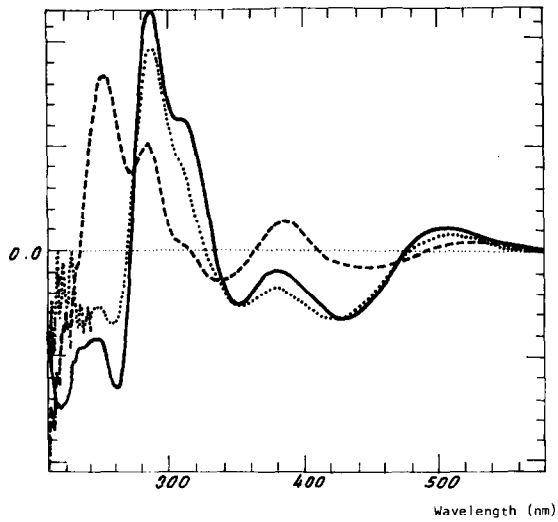


Fig. 6. CD spectra of the first-eluting enantiomers of (dashed line) [6,6′-$\mu$-S(1,7-$C_2B_9H_{10})_2$-2-Co]$^-$ (**9**), (dotted line) [6,6′-$\mu$-O(1,7-$C_2B_9H_{10})_2$-2-Co]$^-$ (**10**) and (solid line) 6,6′-$\mu$-$Me_2$-N(1,7-$C_2B_9H_{10})_2$-2-Co.

maximum of the second peak throughout its descending part contained the second enantiomer in 96–97% purity. The optical purity of the both enantiomers was determined by the HPLC method under discussion.

Following this procedure, about 25 $\mu$g of each enantiomer can be isolated from single injections.

*Circular dichroism spectra*

CD spectra of the first- and second-eluted enantiomers of **1, 4, 5, 7, 9** and **13** prepared according to the scheme described above, exhibit very similar characteristics. The CD spectra of the enantiomers of **1, 4, 5** and **7** with a substituted sulphur bridge are almost identical, differing only in the intensities of the Cotton effects. Fig. 5 exemplifies the CD spectra of the first- and second-eluted enantiomers of **5** and **7**. Similar patterns of CD curves are also exhibited by the parent sulphur- and oxygen-bridged anions (**9** and **10**). CD spectra of their first-eluted enantiomers are depicted in Fig. 6 together with the spectrum of the first-eluting enantiomers of the $Me_2N<$-bridged compound. It can be seen that the orientations of the Cotton curves of these enantiomers are also very similar; only the positions of the Cotton effects are shifted to lower or higher wavelengths.

The close similarities of the CD spectra suggest the same absolute configurations of the enantiomers eluted as the first and second peaks, respectively, for all the compounds studied.

## CONCLUSIONS

$\beta$-Cyclodextrin CSPs have proved to be efficient for the resolution of enantiomers of a variety of deltahedral carborane and metallaborane species, as demonstrated by the present study and by the results reported previously [13,14,16]. So far, 25 enantiomeric pairs of boron chiral species have been resolved on a $\beta$-cyclodextrin column and therefore the number of resolved chiral boranes known before their introduction (about 20) [18] has been more than doubled.

In addition to the study of the enantiomeric discrimination of the above species by use of a $\beta$-cyclodextrin HPLC column, the practical application of such knowledge for preparative purposes has proved possible, enabling us to isolate pure enantiomers in amounts sufficient for subsequent studies of their properties, *e.g.*, CD measurements and possibly for the determination of their structures by X-ray diffraction analysis.

## ACKNOWLEDGEMENTS

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