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DETERMINATION OF THE "ENANTIOMERIC EXCESS" OF HEXAHELICENE AND ITS METHYL-SUBSTITUTED DERIVATIVES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

Resolution of the enantiomers of hexahelicene and several of its derivatives using an improved high-performance liquid chromatographic technique is described. The procedure allows the determination of a less than 0.5% enantiomeric excess with an high accuracy and reproducibility within a short elution time. (20 min for hexahelicene). The results of the resolution of substituted hexahelicenes are discussed. Two types of columns are described which are capable of the partial resolution of helicene enantiomers on a larger scale. The columns are also suitable for the separation of polycyclic aromatic hydrocarbons. This is illustrated by the analysis and separation of a distillation fraction of creosote containing several polycyclic aromatic components.

INTRODUCTION

Hexahelicene (1) is a well known representative of non-planar polycyclic aromatic compounds. It occurs in two enantiomeric forms: P-hexahelicene and M-hexahelicene[1]. Newman *et al.*[2] separated the enantiomers by crystallization with the aid of a special chiral complexing agent, 2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA) (2). Both *R*(−)- and *S*(+)-TAPA form diastereomeric charge-transfer (CT) complexes with the enantiomers of hexahelicene. TAPA, absorbed on silica gel or aluminium oxide, was used with little success in conventional liquid chromatography[3,4]. High-performance liquid chromatography (HPLC) on columns modified with TAPA resulted in an efficient resolution of the enantiomers of carbo- and heterohelicenes[5–8]. These results prompted us to investigate whether the enantiomeric excess (E.E.) of hexahelicene and its methyl-substituted derivatives could be determined by HPLC. Such an analytical method would have facilitated our study of the enantioselective synthesis of hexahelicene in chiral media[9,10].

The determination of E.E. by HPLC is preferred to other techniques like po-

* Dedicated to Professor Dr. Nivard on the occasion of his retirement from the chair of Organic Chemistry at the Catholic University of Nijmegen.

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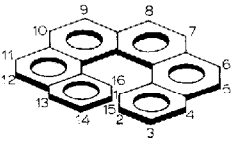

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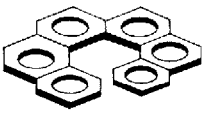

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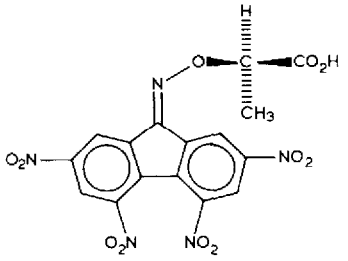

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larimetry, circular dichroism (CD) or NMR spectroscopy because it combines two important features: only very small amounts of a sample are needed, 10 μl of a 10^{-4} *M* solution); impurities, whether optically active or not, do not interfere with the analysis, unless they happen to have the same retention time. These advantages are only valuable if the HPLC analysis fulfils the following conditions: (i) baseline resolution of the enantiomer peaks; (ii) short retention time; (iii) high accuracy and reproducibility; (iv) long lifetime of the modified HPLC column.

The results obtained with TAPA-modified columns[5–8] are promising, but have to be improved to meet our demands. Mikeš *et al.*[6] obtained a resolution factor, *r*, of 1.11 and a resolution, *R*, of 0.75 in the case of hexahelicene with *S*(+)-TAPA coated on silica gel. Numan *et al.*[7] found $r = 1.20$ for hexahelicene, when *R*(−)-TAPA was coated on aluminium oxide. We have chosen silica gel as the stationary phase, because of better specifications concerning the reproducibility of column packing. We prefer the coating technique employed by Numan *et al.*[7] to that used by Mikeš *et al.*[6] since the former is more likely to give a modified stationary phase which is very homogeneous and well reproducible.

Mikeš *et al.*[6] used a large amount of TAPA (1–2 g/10 g of stationary phase). This resulted in a long retention time (45 min), and required a relatively polar mobile phase (20% dichloromethane–cyclohexane). The polar eluent enhanced, however, the bleeding of TAPA from the stationary phase, thus reducing the lifetime of the column. The amount of TAPA has to be reduced to enable the use of a less polar mobile phase, thus minimizing the bleeding and the retention time. The question is whether the resolution of hexahelicene can be achieved on reduction of the amount of the chiral complexing agent. To answer this, several experiments have been done.

EXPERIMENTAL

Apparatus

A Spectra-Physics HPLC system was used: it was made up of a solvent-delivery system (SP8700) equipped with an UV detector (SP8300) operated at 254 nm and a computing integrator (SP4100). Stainless-steel columns (20 or 25 cm × 0.46 cm I.D.) were slurry packed using carbon tetrachloride to suspend the silica particles (LiChrosorb Si 60/5; Merck, Darmstadt, F.R.G.).

A Perkin-Elmer 241 polarimeter was used for the measurement of optical rotations. Spectral measurements were done with a Perkin-Elmer 555 UV–VIS spectrophotometer.

Chemicals

Chiral *R*(−)-TAPA was synthesized according to the method of Block and Newman[11]. The identification of *R*(−)-TAPA has been reported elsewhere[4]. $[\alpha]_D = -91.5°$ (c = 0.64 g/100 ml, chloroform); lit.[11] −97° (c = 0.567 g/100 ml, dioxane).

Hexahelicene and several methyl-substituted derivatives were available from previous studies. Their synthesis, isolation and identification have been described[12–15]. The synthesis and identification of 7,8-dihydrohexahelicene will be elucidated elsewhere; that of 5,6-dihydrohexahelicene has been reported previously[16].

Modification of the stationary phase

The silica gel columns used were tested before they were coated. The columns should show as little tailing as possible, since coating with *R*(−)-TAPA enhances tailing. The test consisted of several experiments with hexahelicene as the sample and diethyl ether–light petroleum (b.p. 60–80°C) (5:95) as the mobile phase. Flaws in the column were revealed in this way. Good columns were eluted for 60 min with 100% toluene (flow-rate 1.5 ml/min). Then the mobile phase was replaced by a solution of *R*(−)-TAPA dissolved in toluene (concentration 10–100 mg/l). A 1-l volume of this solution was recycled over the column for 16 h (flow-rate 1.5 ml/min). The column was then eluted with 15 ml of pure toluene followed by 250 ml of light petroleum (b.p. 60–80°C) containing 5% diethyl ether. The performance of the modified column was tested by injection of racemic hexahelicene.

For the resolution of hexahelicene on a larger scale (*ca.* 5 mg in a single experiment) two types of columns were used: I, a Lobar column (LiChroprep Si 60, Merck, 40–63 μm, 310 mm × 25 mm) modified by recycling a solution of 1 g of *R*(−)-TAPA in 2 l of toluene, during 24 h, flow-rate 2 ml/min, and then eluted with 60 ml of pure toluene and 250 ml of *n*-hexane containing 20% diisopropyl ether; II, a stainless-steel column (250 mm × 7.9 mm I.D.) packed with *R*(−)-TAPA-coated silica gel, prepared by addition of 10 g of LiChroprep Si 60 (15–25 μm) to a solution of 0.7 g *R*(−)-TAPA in 150 ml of ethyl acetate, followed by removal of the solvent *in vacuo*.

RESULTS AND DISCUSSION

The testing of 20 analytical HPLC columns, modified by the procedure described, revealed that the optimum amount of *R*(−)-TAPA for resolution of the enantiomers of hexahelicene is 10–15 mg per g of LiChrosorb Si 60/5 (Fig. 1); this is 10% of the amount applied by Mikeš *et al.*[6]. The mobile phase was diethyl ether–light petroleum (b.p. 60–80°C) (5:95). The columns showed minimum peak tailing and maximum resolution immediately after the coating procedure. However, several days elapsed before the modified column was stable. After stabilization, the resolution was slightly reduced and tailing slightly increased, but the modified stationary phase was completely satisfactory for our analytical purposes. The tailing had increased to a point where it interfered with the desired accuracy of the analysis only after about 500 experiments. One cause of the diminished performance of the coated silica gel was the compression due to the pressure applied; it caused a dead volume at the beginning of the column. This damage could be repaired by filling the dead volume with pellicular silica gel. Use of a less polar eluent to decrease bleeding

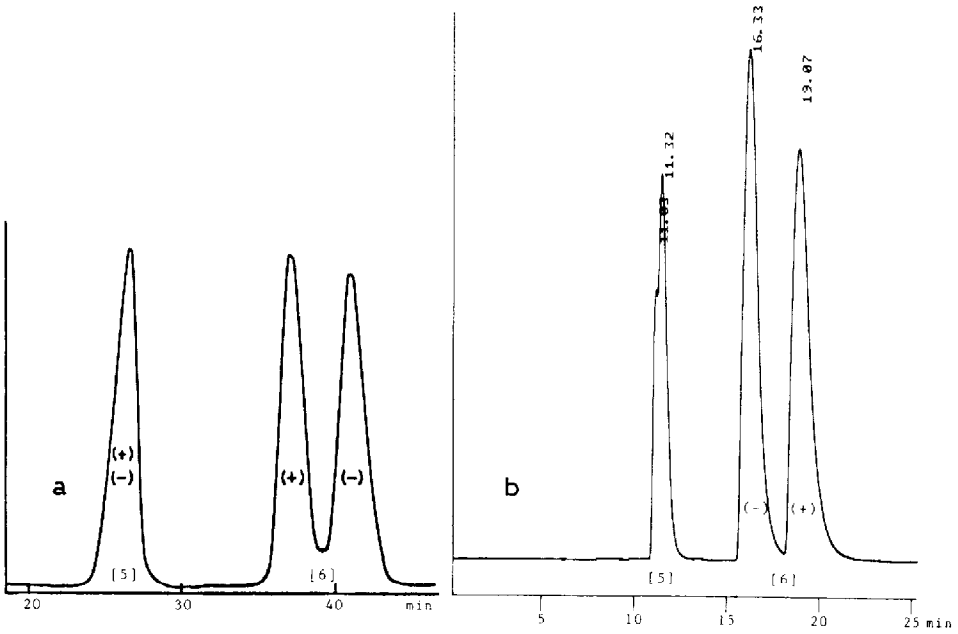


Fig. 1. Comparison of two chromatograms of a mixture of pentahelicene [5] and hexahelicene [6]. (a) As presented by Mikeš *et al.*[6]: column, 110 mg of *S*(+)-TAPA on 1 g of Partisil 7, 2 × 20 cm × 0.23 cm I.D.; mobile phase, 20% dichloromethane–cyclohexane, u = 0.20 cm/s; [5] is not resolved, (−)-[6] is the most strongly retained enantiomer. (b) Column: 15 mg of *R*(−)-TAPA on 1 g of LiChrosorb Si 60/5, (20 + 25 cm) × 0.46 cm I.D. Mobile phase: 5% diethyl ether light petroleum (b.p. 60–80°C); u = 0.18 cm/s; [5] is somewhat resolved, (+)-[6] is the most strongly retained enantiomer. The numbers near the tops of the peak maxima are the retention times (min).

of TAPA from the column could not prevent, long-term damage of the stationary phase by other irreversible processes. Initially, a nearly baseline resolution was achieved with a residual overlap of less than 1% for racemic hexahelicene. After about 500 experiments, however, the overlap had increased to 5% making the column unsuitable for analytical purposes.

The quality of our modified columns is illustrated by the fact that even pentahelicene, which is not resolved by the columns coated with TAPA used by Mikeš *et al.*[6] is partly resolved under our optimized conditions (Fig. 1).

The presence of methyl substituents at the inner positions of hexahelicene (positions 1 and 16) has a large effect on the resolution characteristics. They cause a steric interaction resulting in a less stable CT complex and lower capacity factors (k'), *cf.*, hexahelicene, 1-methyl- and 1,16-dimethylhexahelicene, and the series 3,14-dimethyl-, 1,14-dimethyl- and 1,16-dihexahelicene (Table I). The presence of methyl groups at other positions in hexahelicene results in a stronger CT interaction, reflected in the higher k' and r values of 2-methyl-, 3-methyl-, 2,15-dimethyl-, 3,14-dimethyl- and 4,13-dimethylhexahelicene relative to hexahelicene (Table I).

Mikeš *et al.*[6] found that replacement of the methyl group at the chiral centre of TAPA by a bulkier alkyl group resulted in a decreased resolution. They argued that a good fit in the complex hexahelicene–TAPA was hindered by the presence of

TABLE I

RESOLUTION OF ALKYL-SUBSTITUTED HEXAHELICENES ON SILICA GEL COATED WITH *R*(−)-TAPA

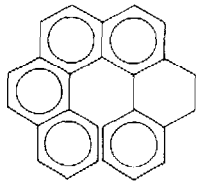
Capacity factor, $k' = (t_R - t_0)/t_0$, where t_R = retention time, t_0 = retention time of non-retained solute; resolution factor, $r = k'_2/k'_1$; resolution, $R = 2(t_{R2} - t_{R1})/(w_1 + w_2)$ where w = bandwidth. Stationary phase: 13 mg of *R*(−)-TAPA on 1 g of LiChrosorb Si 60/5. Mobile phase: diethyl ether–light petroleum (b.p. 60–80°C) (5:95). Linear velocity, u = 0.19 cm/s. Column: 25 cm × 0.46 cm I.D.

Hexahelicene [6]	k'_1	k'_2	r	R
[6]	2.88	3.54	1.23	1.19
1-Methyl-[6]	1.74	2.08	1.20	1.10
2-Methyl-[6]	3.20	4.05	1.27	1.28
3-Methyl-[6]	3.36	4.28	1.27	1.42
1,16-Dimethyl-[6]	0.70	0.79	1.13	0.50
1,14-Dimethyl-[6]	2.18	2.69	1.23	1.32
3,14-Dimethyl-[6]	4.06	5.46	1.34	1.55
2,15-Dimethyl-[6]	3.78	5.05	1.34	1.43
4,13-Dimethyl-[6]	3.83	5.14	1.34	1.48
1,3,14,16-Tetramethyl-[6]	0.75	0.87	1.17	0.70
1,3-Di-*tert.*-butyl-[6]	1.02	1.13	1.11	0.55
Pentahelicene	1.54	1.61	1.04	0.15

such a large group. The group in question should be small enough to fit in the central hole of hexahelicene, but large enough to discriminate between M-hexahelicene and P-hexahelicene. The methyl group of TAPA itself was apparently the most suitable. A similar decrease in resolution is observed when one of the hydrogens at the central positions of hexahelicene is replaced by a larger group.

Comparison of our data for hexahelicene with those given by Mikeš *et al.*[6] (k'_1 = 4.08, r = 1.11, R = 0.75) shows that we have succeeded in improving the resolution of [6], using a less polar mobile phase. In our case k'_1 is reduced to 2.88 giving a shorter retention time. Both r and R are improved.

In Table II the results of the resolution of two dihydrohexahelicenes are given. 5,6-Dihydrohexahelicene (3) is not resolved, although it has a stronger interaction with TAPA than 7,8-dihydrohexahelicene (4) which is resolved by the chiral complexing agent. The different behaviour must be caused by the different positions of the aromatic rings in the hexahelicene skeleton. 5,6-Dihydrohexahelicene consists of a benzene and a benzo[*c*]phenanthrene moiety. 7,8-Dihydrohexahelicene consists of a naphthalene and a phenanthrene moiety. In the CT complex of 5,6-dihydrohexahelicene and TAPA the benzo[*c*]phenanthrene part interacts strongly with TAPA. The benzene moiety has much less interaction, reflected in the absence of any chiral



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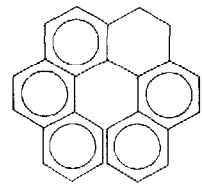

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TABLE II

RESOLUTION OF TWO DIHYDROHEXAHELICENES ON SILICA GEL COATED WITH *R*(−)-TAPA

Conditions and column as in Table I.

Hexahelicene [6]	k'_1	k'_2	r	R
[6]	2.88	3.54	1.23	1.19
5,6-Dihydro-[6]	1.84	1.84	0.00	0.00
7,8-Dihydro-[6]	0.95	1.06	1.12	0.70

recognition. In 7,8-dihydrohexahelicene both aromatic moieties have significant complexing capacity towards the chiral agent, what results in chiral recognition. The complex of 7,8-dihydrohexahelicene and TAPA is weaker than that of 5,6-dihydrohexahelicene and TAPA. It seems that for a successful chiral discrimination by *R*(−)- or *S*(+)-TAPA the aromatic moieties must be divided more or less equally over both halves of the helicene skeleton, as is reflected in the data in Table II.

Determination of the "enantiomeric excess" of hexahelicene

As described above the aim of this investigation was to develop an HPLC method for the determination of the E.E. values of hexahelicene obtained upon irradiation of the proper hexahelicene precursor in a chiral medium. E.E. values obtained in previous experiments were of the order of 1%[9,10,17–19]. Therefore the accuracy of the HPLC analysis has to be better than 0.5% to be of value for this purpose. The resolution of the enantiomers of hexahelicene could be improved by using two coupled modified columns. Fig. 2 shows the chromatogram of a sample of racemic hexahelicene. It reveals a small overlap of the peaks of the enantiomers of hexahelicene, calculated to be 0.5 ± 0.3%. Tailing of the peaks interferes with the accuracy of the integration, due to a poor signal-to-noise ratio in the tail of the peak. Therefore the value of the peak threshold has to be determined carefully. When this value is too low or too high it leads to a systematic error. To prevent such errors the system has to be calibrated with racemic hexahelicene. The standard procedure for

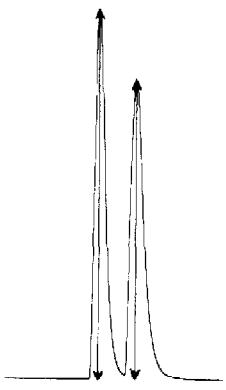

Fig. 2. Chromatogram of racemic hexahelicene showing the peak maxima and the overlap of the enantiomers. Conditions and stationary phase as in Fig. 1b.

our determination of the E.E. value of hexahelicene consisted of three experiments with the sample of unknown E.E., and two with a racemic sample to determine the correction for peak overlap. In this way the E.E. value of an unknown sample could be determined with an accuracy of 0.5%. When the sample shows an E.E. value of less than 1%, an experimental error of 0.5% is too large for useful analysis. In such a case the accuracy of the HPLC analysis can be improved by raising the number of experiments. For practical reasons this increase was generally restricted to fifteen, reducing the experimental error statistically to 0.3%.

When a sample has an E.E. value of less than 0.5% the E.E. value is difficult to determine by integration of the peak areas. In such cases, correct information about the E.E. value can be deduced from the ratio of the peak heights of the enantiomers. This ratio is sensitive to small variations in E.E. value (Fig. 2). The question of whether P-hexahelicene or M-hexahelicene is the enantiomer in excess in a sample is answered by comparing the ratio of the peak maxima to that found for a racemic sample.

Fig. 3 shows the HPLC analysis of a typical sample of enantiomerically enriched hexahelicene obtained by irradiation of the hexahelicene precursor in a chiral medium. The chromatogram shows that two side-products have been formed, identified as 5,6- and 7,8-dihydrohexahelicene. The presence of these compounds in the sample prevents an accurate determination of the E.E. values by polarimetry or CD,

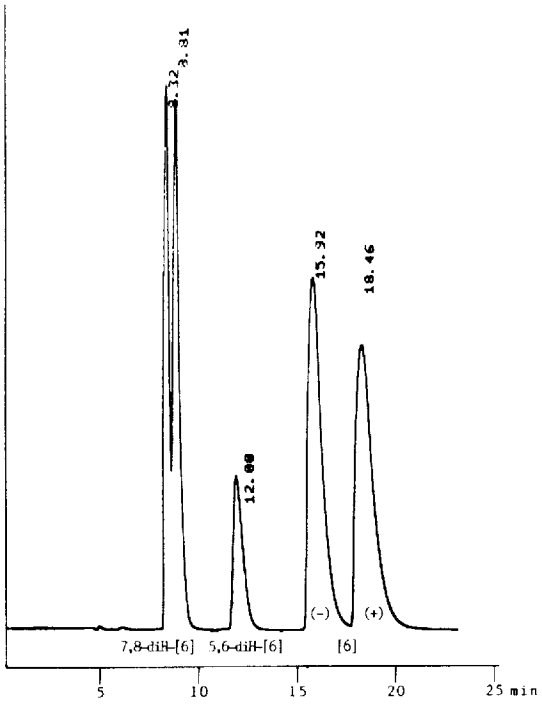

Fig. 3. Chromatogram of a mixture of 5,6-dihydrohexahelicene(5,6-diH-[6]), 7,8-dihydrohexahelicene (7,8-diH-[6]) and hexahelicene [6]. Column: 15 mg of *R*(−)-TAPA on 1 g of LiChrosorb Si 60/5, (20 + 25 cm) × 0.46 cm I.D. Mobile phase: diethyl ether–light petroleum (b.p. 60–80°C) (5:95); u = 0.19 cm/s.

TABLE III

SPECIFIC ROTATION OF HEXAHELICENE DETERMINED FROM ENANTIOMERICALLY ENRICHED SAMPLES OF HEXAHELICENE

α_D = Measured optical rotation; $[\alpha]_D$ = specific rotation of sample; $[\alpha]_D$ of hexahelicene = specific rotation of hexahelicene (E.E. = 100%); wavelength = 589 nm; solvent = chloroform; temperature = 25°C.

α_D (°)	*Concentration (g/100 ml)*	$[\alpha]_D$ (°)	*E.E. (%)*	$[\alpha]_D$ *of hexahelicene* (°)
+0.442	0.0151	+2930	82.5	+3550
+0.767	0.0349	+2200	59.2	+3710
−0.263	0.0374	− 705	19.3	−3645
−0.643	0.0282	−2280	60.4	−3775
−0.597	0.0206	−2900	76.9	−3770

but our HPLC analysis appears a reliable method of obtaining an accurate E.E. value.

In the final part of this paper several applications of HPLC on silica gel coated with *R*(−)-TAPA are discussed.

Separation of the enantiomers of methyl-substituted hexahelicenes and determination of their specific rotation

In order to study the specific rotations of methyl-substituted hexahelicenes such helicenes have to be separated into enantiomers. For an accurate measurement of the optical activity, samples of about 1 mg are needed. The collection of such an amount by HPLC on an analytical column is very time-consuming, we therefore chose a large-scale procedure. We modified a Lobar column LiChroprep Si 60 (particle size 40–63 μm; 31 cm × 2.5 cm; Merck, Darmstadt, F.R.G.) as described above. In order to test the method, racemic hexahelicene was separated; a sample of 10 mg dissolved in 3 ml of diisopropyl ether–hexane (40:60) was injected on the Lobar column by means of a loop. The mobile phase was diisopropyl ether–hexane (5:95), flow-rate 2.5 ml/min. The fractions (each 10 ml) were analysed by HPLC. It appeared that the mixture was only partly resolved. The enriched fractions were concentrated and reinjected on the Lobar column. In this way a set of fractions was obtained having E.E. values from 75% for M-hexahelicene to 80% for P-hexahelicene. The optical rotations of the fractions varied from 0.3 to 0.8° (± 0.003°). The concentration of hexahelicene in the fractions was determined by UV spectroscopy; that of the third sample (E.E. = 19.3%), the only fraction which was large enough to crystallize, by weighing. From these data the specific rotations were calculated (Table III); standard deviation 95°. The mean value of the specific rotation for hexahelicene is 3690 ± 45°, in excellent agreement with that obtained by Newman *et al.*[2] (3675 ± 35°).

Encouraged by this success we repeated the procedure for 1-methyl-, 2-methyl- and 3,14-dimethylhexahelicene. The mean values of the specific rotations of these compounds together with their standard deviations are collected in Table IV. Chromatograms of enriched samples are shown in Fig. 4.

The Lobar column failed to separate the enantiomers of 1,16-dimethylhexahelicene. Examination of the data collected in Table I shows that 1,16-dimethylhexahelicene is the methyl-substituted hexahelicene which is least resolved by *R*(−)-

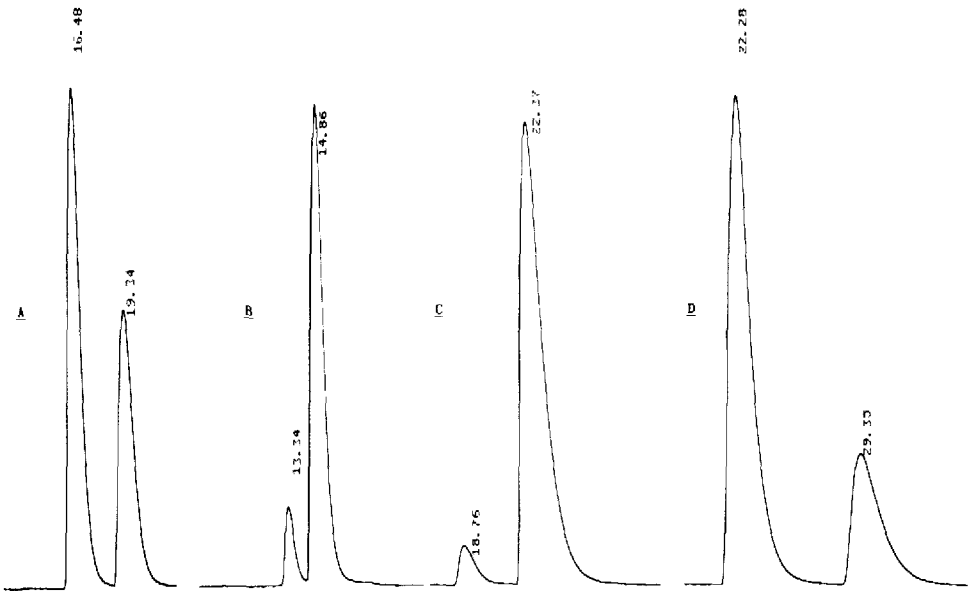

Fig. 4. Chromatograms of enriched samples of helicenes. (A) (−)-Hexahelicene; (B) (+)-1-Methylhexahelicene; (C) (+)-2-Methylhexahelicene; (D) (−)-3,14-Dimethylhexahelicene. Conditions as in Fig. 1b. Retention times in min.

TAPA. Therefore, we changed to a finer type of silica gel (15–25 μm) and used another coating technique (see Experimental section). A 5-mg amount of 1,16-dimethylhexahelicene was dissolved in 2 ml of diisopropyl ether–hexane (2:98) and injected on the column by means of a loop. The mobile phase was 2% diisopropyl ether–hexane (flow-rate 2 ml/min). Fractions of 4 ml were collected and analysed by HPLC to measure the E.E. value. The new column gave very good results. The highly enriched fractions (E.E. > 80%) were collected and reinjected on the column. After two experiments the E.E. value was over 95% (Fig. 5). Each experiment took about half an hour. After repeating this procedure several times the portions of highly enriched 1,16-dimethylhexahelicene, (+) as well as (−), were large enough to crystallize. The concentration of the highly enriched sample could be determined by weighing. The specific rotation of 1,16-dimethylhexahelicene could then be evaluated. The enantiomers of 3-methyl-, 1,14-dimethyl-, 2,15-dimethyl-, 1,3,14,16-tetramethyl-,

TABLE IV

SPECIFIC ROTATIONS OF METHYL SUBSTITUTED HEXAHELICENES

$[\alpha]_D$ = Specific rotation of helicene (E.E. = 100%); other details as in Table III.

Hexahelicene [6]	$[\alpha]_D$ (°)
[6]	3690 ± 45
1-Methyl-[6]	3345 ± 40
2-Methyl-[6]	3790 ± 30
3,14-Dimethyl-[6]	3670 ± 40

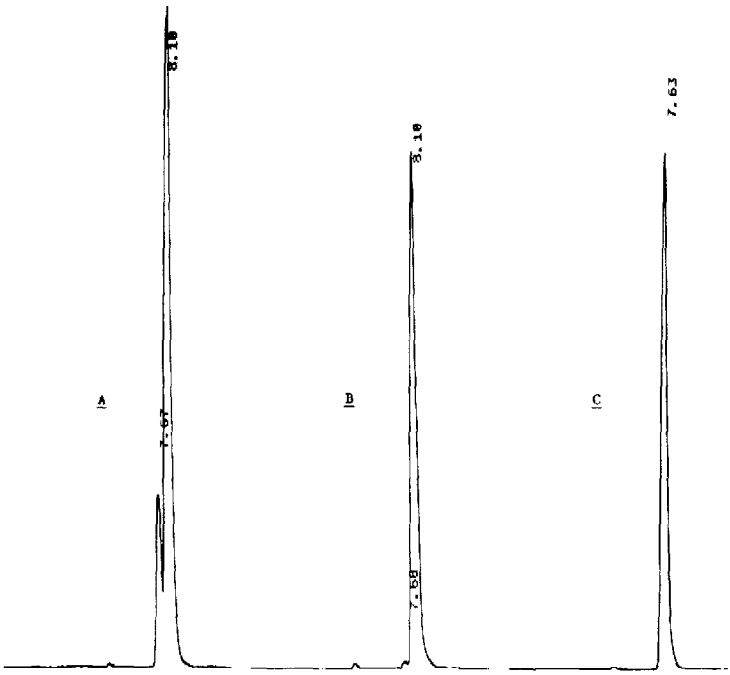

Fig. 5. Chromatograms of enriched samples of 1,16-dimethylhexahelicene. (A) (+)-1,16-Dimethylhexahelicene obtained after one elution on LiChroprep Si 60 (15–25 μm) coated with $R(-)$-TAPA (70 mg on 1 g of LiChroprep). (B) After two elutions on the same stationary phase. (C) (−)-1,16-Dimethylhexahelicene obtained after two elutions on the same stationary phase. Column: 15 mg of $R(-)$-TAPA on 1 g of LiChrosorb Si 60/5, (20 + 20 + 25 cm) × 0.46 cm I.D. Mobile phase diethyl ether–light petroleum (b.p. 60–80°C) (5:95); u = 0.24 cm/s. Retention times in min.

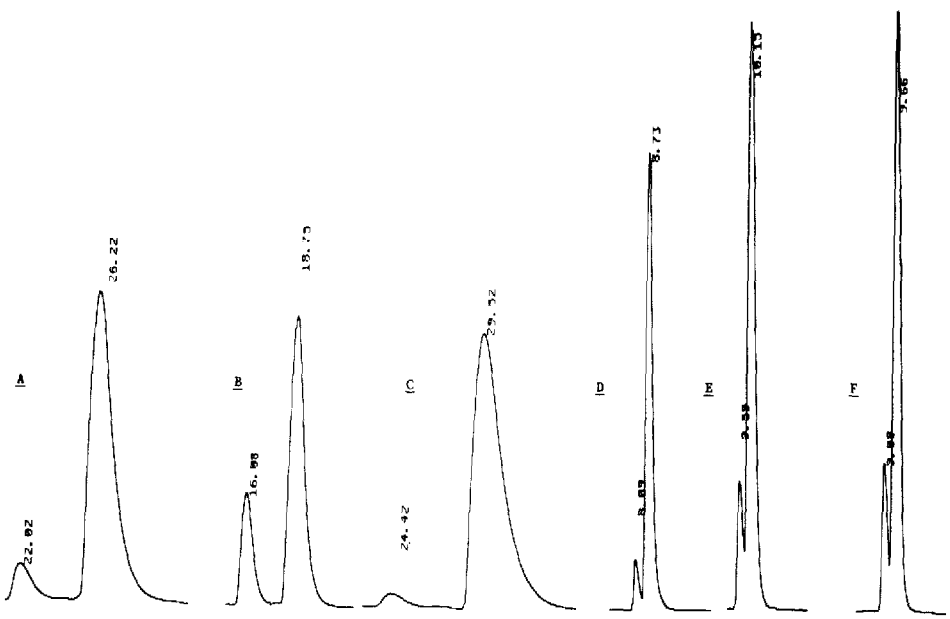

Fig. 6. Chromatograms of enriched samples of helicenes. (A) (+)-3-Methylhexahelicene; (B) (+)-1,14-dimethylhexahelicene; (C) (+)-2,15-dimethylhexahelicene; (D) (+)-1,3,14,16-tetramethylhexahelicene; (E) (+)-1,3-di-*tert*.-butylhexahelicene; (F) (+)-7,8-dihydrohexahelicene. Conditions and stationary phase as in Fig. 5.

TABLE V

SPECIFIC ROTATIONS OF SEVERAL DERIVATIVES OF HEXAHELICENE

Details as in Table IV.

Hexahelicene [6]	$[\alpha]_D$ (°)
3-Methyl-[6]	3190 ± 40
1,14-Dimethyl-[6]	3350 ± 40
1,16-Dimethyl-[6]	3040 ± 60
2,15-Dimethyl-[6]	3990 ± 50
1,3,14,16-Tetramethyl-[6]	3070 ± 40
1,3-Di-*tert.*-butyl-[6]	2740 ± 60
7,8-Dihydro-[6]	1790 ± 40

1,3-di-*tert.*-butyl- and 7,8-dihydrohexahelicene were separated by the same procedure (Fig. 6). The specific rotations of these compounds are collected in Table V.

The separation of several derivatives of hexahelicene

The applications of a stationary phase coated with TAPA are not restricted to the separation of enantiomers. Several difficult purifications are easily achieved by means of this modified silica gel. Irradiation of 2,7-bis(*m*-methylstyryl)naphthalene gives a mixture of 1,16-, 1,14- and 3,14-dimethylhexahelicene, which is difficult to

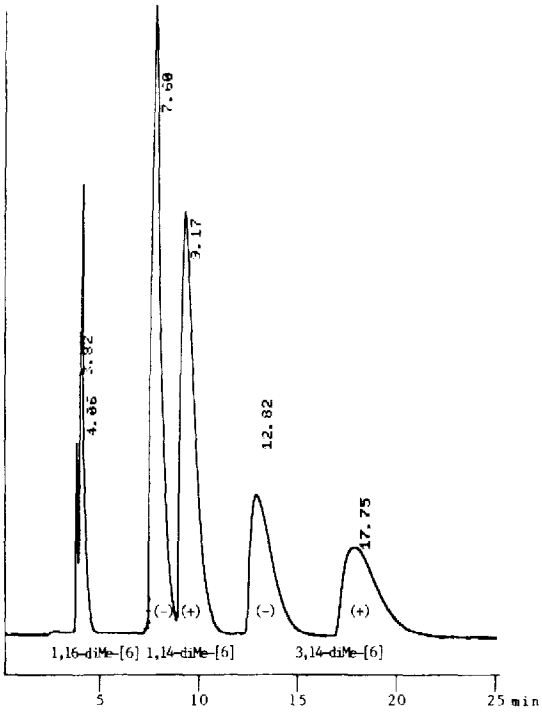

Fig. 7. Chromatogram of a mixture of 1,16-, 1,14- and 3,14-dimethylhexahelicene (1,16-diMe-[6], 1,14-diMe-[6] and 3,14-diMe-[6], respectively). Column: 15 mg of *R*(−)-TAPA on 1 g of LiChrosorb Si 60/5, 20 cm × 0.46 cm I.D. Mobile phase: diethyl ether–light petroleum (b.p. 60–80°C) (5:95); u = 0.15 cm/s.

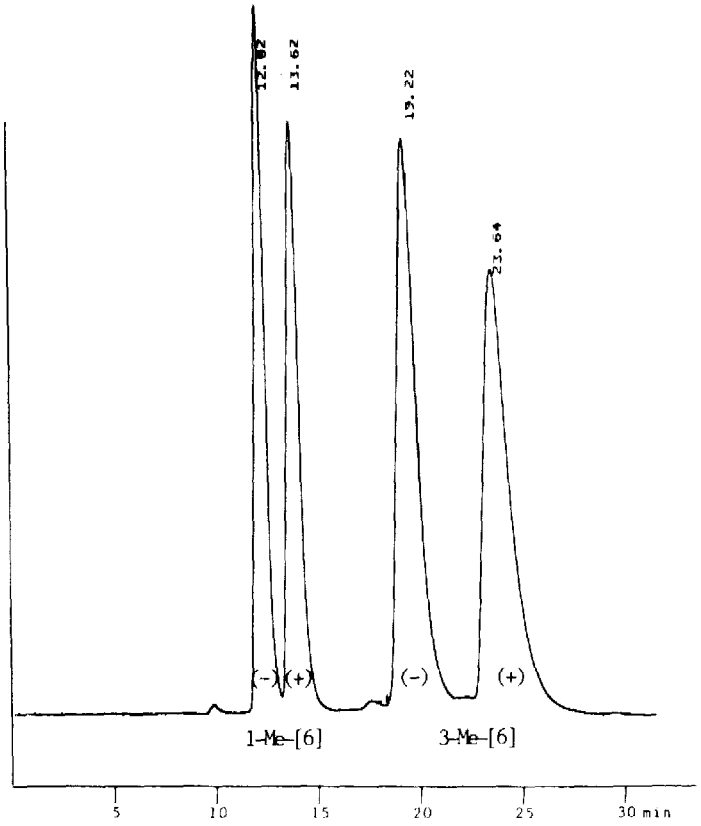

Fig. 8. Chromatogram of a mixture of 1-methylhexahelicene (1-Me-[6]) and 3-methylhexahelicene (3-Me-[6]). Column: 15 mg of *R*(−)-TAPA on 1 g of LiChrosorb Si 60/5, (20 + 25 cm) × 0.46 cm I.D. Mobile phase: diethyl ether–light petroleum (b.p. 60–80°C) (5:95); u = 0.17 cm/s.

separate on conventional materials[14]. Fig. 7 shows the chromatogram of a mixture of 1,16-, 1,14- and 3,14-dimethylhexahelicene on silica gel coated with *R*(−)-TAPA. The same column as applied to the resolution of 1,16-dimethylhexahelicene was used to separate this mixture. The amount of sample that can be separated in one experiment depends on the solubilities of the components in the mobile phase: diisopropyl ether–hexane (5:95). A loop of 5 ml was used for this chromatographic separation; by which about 10 mg of the mixture were injected on the column.

Fig. 8 shows the chromatogram of a mixture of 1-methyl- and 3-methylhexahelicene obtained by irradiation of 2-(*m*-methylstyryl)benzo[*c*]phenanthrene[13]. This mixture is easily separated by the same chromatographic procedure described for the mixture of 1,16-, 1,14- and 3,14-dimethylhexahelicene.

Fig. 3 shows the chromatogram of a mixture of hexahelicene, 5,6- and 7,8-dihydrohexahelicene, obtained by irradiation of 2-styrylbenzo[*c*]phenanthrene[12,16]. The separation was realized on the same column and under the same conditions as in the case of the mixture of 1,16-, 1,14- and 3,14-dimethylhexahelicene.

TABLE VI

CHROMATOGRAPHIC DATA FOR NON-CHIRAL POLYCYCLIC AROMATIC COMPOUNDS ON SILICA GEL COATED WITH *R*(−)-TAPA

Column: 15 mg of *R*(−)-TAPA on 1 g of LiChrosorb Si 60/5, 20 cm × 0.46 cm I.D. Mobile phase: diethyl ether–light petroleum (b.p. 60–80°C) (5:95); u = 0.19 cm/s.

Compound	*Capacity factor*
Benzene	0.10
Naphthalene	0.33
Acenaphthylene	0.64
Phenanthrene	1.31
Anthracene	1.86
Pyrene	2.24
Fluoranthene	3.66
Chrysene	15.00
Benzo[*a*]pyrene	25.35
Perylene	31.02
Coronene	>35.00

The separation of achiral polycyclic aromatic compounds

Chromatographic data for several common non-chiral polycyclic aromatic compounds on a column modified with *R*(−)-TAPA are collected in Table VI. The data demonstrate clearly that *R*(−)-TAPA coated on silica gel is an efficient selector for polycyclic aromatic compounds. This knowledge was applied successfully to the identification of the unknown components of a fraction obtained by distillation from creosote. Creosote is used for the preservation of wood and contains a complex mixture of polycyclic aromatic compounds. The particular fraction showed volatile mutagenic activity[20]. It contained a mixture of unidentified compounds. The column and method described above was used: LiChroprep Si 60 (15–25 μm) coated with *R*(−)-TAPA (70 mg on 1 g of LiChroprep Si 60); diisopropyl ether–hexane (2:98) as eluent. A loop of 1 ml was used to inject the sample. It contained about 10 mg of sample. Pyrene and fluoranthene proved to be the main components of the sample, the latter having been identified as the volatile mutagenic compound present in creosote[21].

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