CHROM. 10,746

RESOLUTION OF OPTICAL ISOMERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

A COMPARISON OF TWO SELECTOR–SELECTAND SYSTEMS

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SUMMARY

The chiral selectivities of two optically active selectors, binaphthyl-2,2'-diyl hydrogen phosphate (binaphthylphosphoric acid, BPA) and 2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA), are compared. Whereas all carbo- and heterohelicene selectands can be resolved on TAPA columns, only helicenes containing heteroatoms or electron-donating substituents could be easily separated on BPA. Resolution factors (r) for several sulphur-, bromine- and nitrogen-containing helicenes on coated and linked BPA and TAPA columns are given. The highest resolution factor was obtained for 9,10-diaza(7,8;11,12)dibenzo-heptahelicene ($r = 1.60$) on a TAPA column silanized with N-2-(aminoethyl)amino-propyl and methyl groups (1:4) before coupling with the selector. Lowering the column temperature to 2° allowed the partial resolution of some carbohelicenes on BPA. Steric and binding requirements for chiral recognition between selectors and selectands and the influence of environmental changes (spacer length, mobile phase, mode of selector application) on the resolution process are discussed.

INTRODUCTION

We have previously reported[1,2] the introduction of the chiral selector* 2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA) and its homologues for the resolution of carbohelicene selectands*. Since then, other workers have shown that heterohelicenes[3] and a dissymmetric olefin[4] can also be resolved on TAPA-coated columns using high-performance liquid chromatography (HPLC).

We now report the use of a new chiral selector, binaphthyl-2,2'-diyl hydrogen phosphate (binaphthylphosphoric acid, BPA), for the chiral recognition of helicenes, especially those which contain heteroatoms or electron-donating substituents (for a preliminary communication, see ref. 5), and to compare the selectivity of the TAPA and BPA selectors.

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* Selector and selectand are cybernetic terms for resolving agent and sample input, respectively. These terms were introduced by us at the 11th International Symposium on Chromatography in Birmingham, 1976[2].

TAPA[6] (Fig. 1a) is a chiral charge-transfer (CT) complexing agent in which the strong electron-accepting tetranitrofluorenylidene moiety provides the binding power and the oxypropionic acid moiety provides the chiral selectivity for electron-donating helicene* selectands.
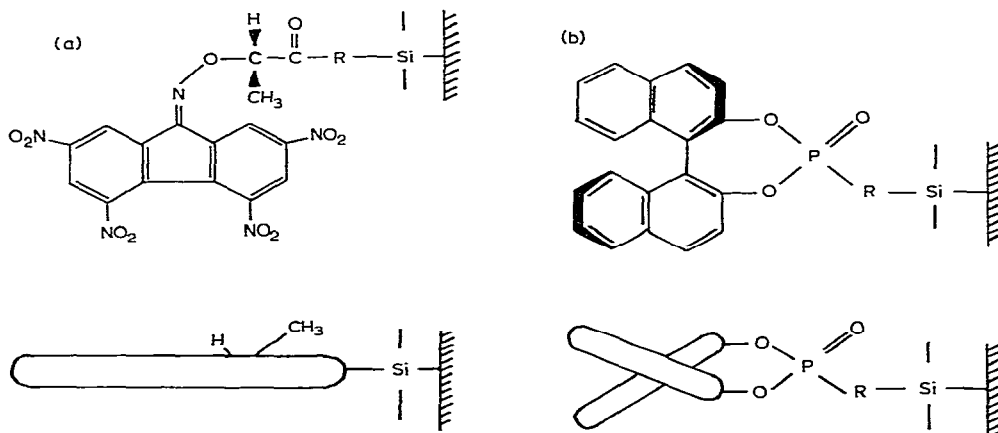

Fig. 1. Top and side views of two selectors for the chiral recognition of helicenes. (a) S(+) TAPA; (b) M(−) BPA, both covalently linked to microporous silica particles. R = NH(CH₂)₃ or NH(CH₂)₂NH(CH₂)₃.

Following our successful results with TAPA, we sought other selectors that might be more suitable for the chiral recognition of selectands with carbon or point asymmetry. We have previously proposed that interpenetration of one molecule into a cavity of another, or topographical complementary convexity and concavity of the selector and selectand, may be necessary for chiral recognition. In the TAPA-helicene system, the former possesses chiral convex and the latter chiral concave topography. For the recognition of point asymmetrical selectands, a selector of the opposite, i.e., chiral concave topography, should therefore be required.

The chiral, cyclic, atropisomeric compound BPA, first synthesized by Mar-schalk in 1928[7], was shown by Jacques and Fouquey[8] to be an effective resolving agent for aromatic amines by preferential ion-pair crystallization with one BPA enantiomer. This compound appeared to be a good candidate for an HPLC selector of point chiral selectands. Chromatographic studies of the interactions between BPA and aromatic amines are now in progress and will be reported later.

In this paper we discuss chiral recognition between the chiral binaphthyl moiety of BPA and the helicenes without involving ion-pair binding. In its interactions with helicenes, the naphthyloxo moieties of BPA are weak electron acceptors (π acids), whereas the chiral binaphthyl provides for the chiral selectivity. In BPA the chirality is determined by axial symmetry in the molecule, in contradistinction to the carbon asymmetry in TAPA (Fig. 1).

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* Helicenes contain *ortho*-condensed benzene rings, are inherently chiral, having a two-fold symmetry axis C_2, perpendicular to their cylindrical helixes. They possess the highest optical rotations yet reported for organic molecules[1]. Helicene nomenclature is established by the "helicity rule". If the helix is left-handed, it is designed "minus" and denoted by M, if it is right-handed, by "plus" or P. The prefixes (+) and (−) indicate the sense of the optical rotation of the compound.

EXPERIMENTAL

Apparatus

A Pye Unicam Separator 20 (Pye Unicam Ltd., Cambridge, Great Britain) equipped with a 254-nm UV detector was used. Small stainless-steel columns (20 × 0.23 cm I.D.) were slurry packed using carbon tetrachloride to suspend the neat or bonded silica particles (Partisil 5; Whatman, Springfield Mill, Kent, Great Britain).

Optical rotations were measured on a Kreispolarimeter 0.01° (Carl Zeiss, Oberkochen/Württemberg, G.F.R.) and mass spectra with an LKB 2091 gas chromatograph–mass spectrometer (LKB, Bromma, Sweden).

Chemicals

Chemicals for the synthesis of TAPA were listed earlier[2]. Chemicals for the synthesis of BPA included bi-2-naphthol, phosphorus oxychloride, cinchonine and cinchonidine (all from Fluka, Buchs, Switzerland). Reagents for linked phases included methyltriethoxysilane (Fluka), 3-aminopropyltriethoxysilane and N-2-(aminoethyl)-3-aminopropyltrimethoxysilane (Union Carbide, New York, U.S.A.). The helicenes were kindly provided by Prof. R. H. Martin (Brussels, Belgium), Prof. H. Wynberg (Groningen, The Netherlands) and Prof. H. Rau (Stuttgart-Hohenheim, G.F.R.).

Synthesis of chiral selectors

Chiral R(−) and S(+) TAPA were synthesized according to the method of Block and Newman[9] and chiral P(+) and M(−) BPA according to Jacques and Fouquey[8].

The identification of TAPA and BPA enantiomers by polarimetry, microanalysis, mass spectrometry, NMR chiral shift (for BPA) and melting-point determinations have been reported elsewhere[2,5].

Bonded and in situ coated chiral selectors

TAPA and BPA selectors were in situ coated or covalently linked to microsilica particles as described earlier[2]. In addition, one linked TAPA phase was prepared by first silanizing the microsilica particles with N-2-(aminoethyl)-3-aminopropyltrimethoxysilane and methyltriethoxysilane in a molar ratio of 1:4 before coupling with TAPA.

Modification of selectands

Hexa- and heptacarbohelicenes were brominated in carbon tetrachloride at 70° for 2 h. Mass spectra of the brominated helicenes indicated that they were dibrominated ($M^{+\cdot}$, $M^{+\cdot} + 2$, $M^{+\cdot} + 4 = 484$, 486, 488 and 534, 536, 538, respectively).

RESULTS AND DISCUSSION

Examination of space-filling CPK (Corey–Pauling–Koltun) models of BPA or TAPA and the helicenes show a good topographical fit between either selector and the selectands (carbo- or heterohelicenes). However, chromatographic examination of the molecular interactions between the two selectors and the carbohelicenes showed good resolutions only with the TAPA selector[1,2]. Only small resolution effects (with

a shoulder preceding the main peak) were observed for the [7]-, [13]-, and [14]-carbohelicenes (resolution factors $r = 1.04$, 1.03 and 1.04, respectively) on a linked BPA column at room temperature.

The first selectands with which significant resolution effects were observed on BPA columns were two helicenes containing bromine and nitrogen atoms, respectively: 8,20-dibromodiphenanthro(4,3-a; 4',3'-j)chrysene[10] (I) and 9,10-diaza(7,8; 11,12)dibenzoheptahelicene[11] (II) (Fig. 2a). It can be seen that nearly baseline reso-

I   II
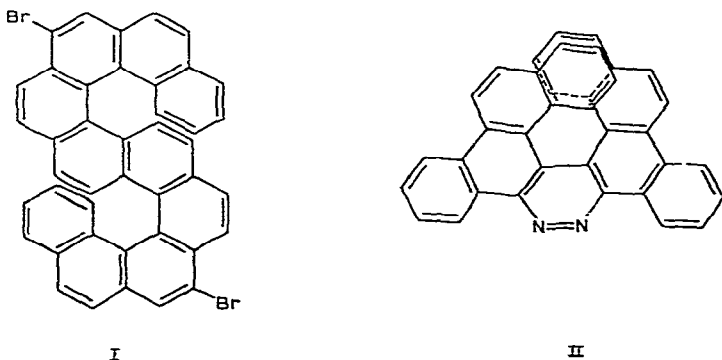
lution of these two helicenes was achieved, whereas no resolution effect was observed with [12]helicene, in contrast to the good resolution of all three helicenes on a TAPA column (Fig. 2a).
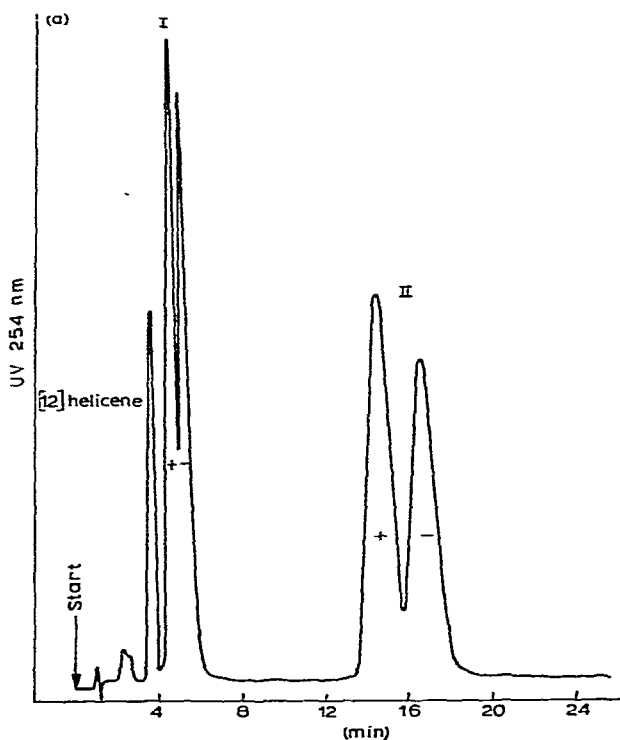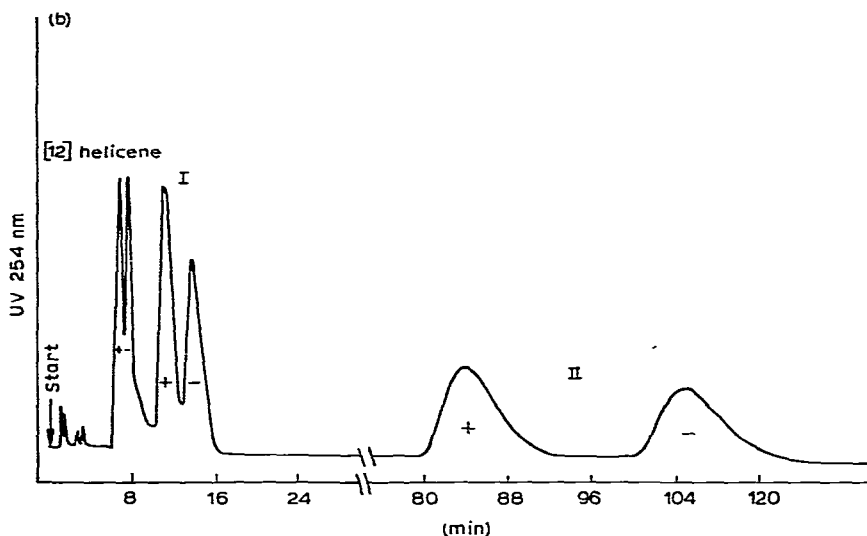

Fig. 2 (a)

Fig. 2. Chromatogram of dodeca-, dibromo double (I) and diazadibenzoheptahelicene (II) on two chiral selectors. (a) On 20% M(−) BPA (α_D^{23} = −480°, ethanol)–NH(CH$_2$)$_3$Si–Partisil 5, 20 × 0.23 cm I.D., mobile phase 5% methylene chloride–n-hexane, flow-rate 1 ml/min. (b) On 20% S(+) TAPA (α_{633}^{23} = +85°, chloroform)–NH(CH$_2$)$_3$Si–Partisil 5, 20 × 0.23 cm I.D., mobile phase 50% methylene chloride–n-hexane, flow-rate 1 ml/min.

We then analysed two sulphur-containing heterohelicenes, dinaphtho(1,2-d; 1′,2′-d′)benzo(1,2-b; 4,3-b′)dithiophene[12] (III) and dithieno(3,2-e; 3′,2′-e′)benzo(1,2-b; 4,3-b′)bis(1)benzothiophene[12] (IV), which show resolution factors on a linked TAPA
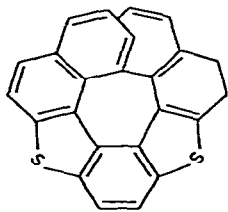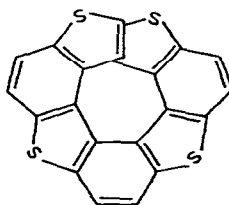
column (Fig. 3b, Table I) similar to those of the [6]- and [5]carbohelicenes, respectively[2]. On BPA, compound III also shows a resolution effect, although smaller than on the TAPA column, whereas only a slight shoulder could be detected for compound IV (Fig. 3a). For complete resolution of these heterohelicenes on BPA, about four and nine recyclings[1], respectively, would be required.

These initial results strongly indicated the requirement of electron-donating atoms in the helicenes for good resolution on BPA. To test this hypothesis further, we decided to brominate the [6]- and [7]carbohelicenes. Mass spectra of the derivatized helicenes showed that they had been dibrominated. Chromatography of the dibromo[6]- and [7]helicenes on BPA gave good resolutions (Fig. 4), intermediate between those of the sulphur-containing heptahelicenes and the dibromo double and diaza helicenes (Table I).
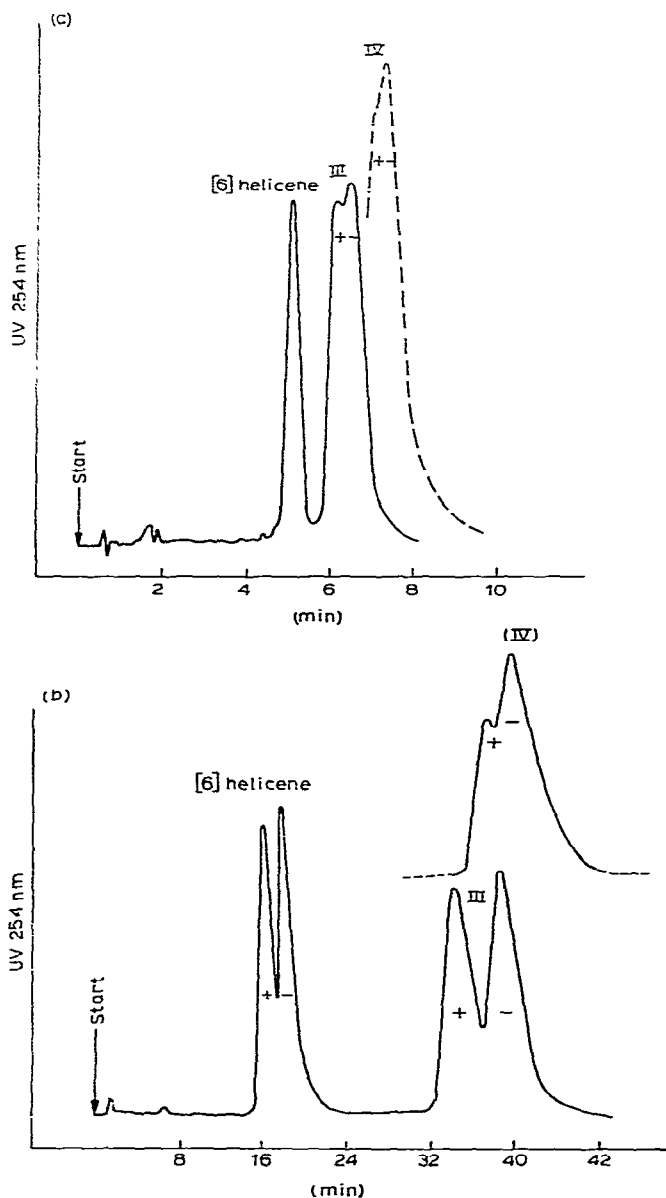
Fig. 3. Chromatogram of hexa-, disulphur hepta- (III) and tetrasulphur heptahelicenes (IV). (a) On linked M(—) BPA, mobile phase *n*-hexane, other conditions as in Fig. 2a. (b) On linked S(+) TAPA–NH(CH₂)₃Si–Partisil 5, 20 × 0.23 cm I.D., mobile phase 10% methylene chloride–*n*-hexane, flow-rate 1.5 ml/min.

Environmental variations

The effects of environmental changes on the resolution process were studied by comparing linked and coated columns (Table I), and by varying the mobile phase, temperature and spacer length on linked selectors.

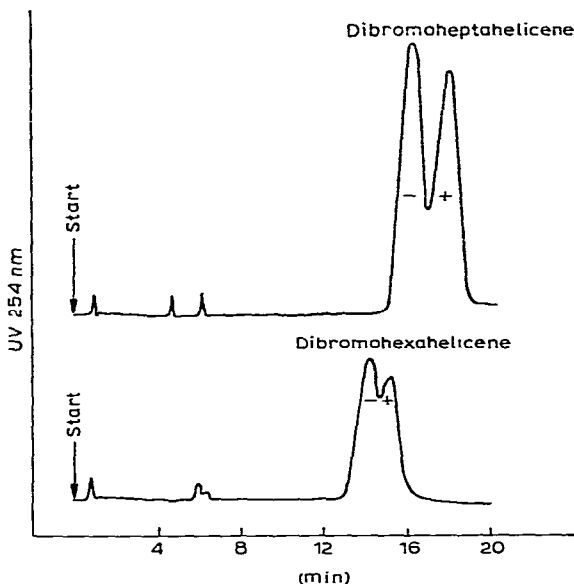Fig. 4. Resolution of dibromohexa- and dibromoheptahelicene on BPA. Chromatographic conditions: 12% P(+) BPA $(\alpha_D^{23} = +525°$, methanol) coated on Partisil 5, 20 × 0.23 cm; mobile phase 2% methylene chloride–n-hexane; flow-rate 1 ml/min.

On TAPA-coated silica gel columns the resolution of the higher carbohelicenes was better, but that of the lower helicenes was slightly worse, than on a bonded TAPA column linked through an aminopropyl group[2]. The heterohelicenes examined also showed improved resolution factors on the linked TAPA column. In contrast, the coated BPA column gave slightly higher resolution factors for the brominated and diaza helicenes, but lower values for the sulphur heterohelicenes, than on a BPA column linked through the aminopropyl spacer.

Small improvements in r values were observed when changing from cyclohexane to n-hexane and from methylene chloride to chloroform in the mobile phase. However, slightly more tailing was observed when using chloroform instead of methylene chloride.

Two additional linked BPA columns were prepared. Firstly, a longer spacer, $NH(CH_2)_2NH(CH_2)_3Si$, was incorporated between the silica gel and BPA. On this column the resolution factors for the helicenes were lower (e.g., $r = 1.12$ for compound I) than with the shorter spacer. Secondly, a mixed bonded phase was prepared by first silanizing silica gel with methyl and 3-aminopropyl silanizing agents in a molar ratio of 9:1 before coupling with BPA. The resulting column showed no resolution effects for any of the helicenes.

A mixed bonded TAPA column (Table I) was also prepared in which the silica gel was initially silanized with methyl and N-2-(aminoethyl)-3-aminopropyl groups in a molar ratio of 4:1 before coupling with TAPA. A dramatic improvement in the resolution of the diazahelicene was observed with an increase in r from 1.26 to 1.60 compared with the 3-aminopropyl-linked column. This is the highest resolution factor that we have yet observed for the helicenes. On the other hand, the r values for the other helicenes were slightly lower than on the short spacer.

TABLE I

RESOLUTION OF HELICENES ON CHIRAL BPA AND TAPA SELECTORS

A comparison is given of the resolution of some carbo-, hetero- and bromohelicenes on linked and coated BPA and TAPA columns. (1) 20% M(−) BPA–$NH(CH_2)_3Si$–Partisil 5; flow-rate 1 ml/min. (2) 12% P(+) BPA coated on Partisil 5, flow-rates of 1.0, 1.5, 2.0 ml/min with mobile phases of 0, 5 and 10% methylene chloride in n-hexane, respectively. (3) 20% S(+) TAPA–$NH(CH_2)_3Si$–Partisil 5. (4) 10% S(+) TAPA coated on Partisil 5. (5) 15% S(+) TAPA–$NH(CH_2)_2NH(CH_2)_3Si$–Partisil 5, mixed linked phase with 80 mole-% of the silica hydroxyl groups silanized with CH_3Si groups. Flow-rates with columns 3, 4 and 5 were 1.5 ml/min with the less polar, and 1.0 ml/min with the more polar, mobile phase. The temperature for all columns was 23–25°.

Selectand	M(−) BPA (linked)			P(+) BPA (coated)			S(+) TAPA (linked)			S(+) TAPA (coated)			S(+) TAPA (mixed linked)		
	m.ph.*	k'	r	m.ph.*	k'	r	m.ph.*	k'	r	m.ph.*	k'	r	m.ph.*	k'	r
[6]Carbohelicene	0:1	3.73	—	0:1	7.63	—	1:9	17.11 19.00	1.11	1:9	10.81 12.00	1.11	1:9	9.00 9.60	1.07
[7]Carbohelicene	0:1	4.27 4.45	1.04	0:1	8.88 9.27	1.04	1:9	26.56 30.33	1.14	1:9	18.44 20.75	1.13	1:9	13.20 14.50	1.10
Disulphur[7]helicene (III)	0:1	4.73 4.96	1.05	0:1	9.16 9.49	1.04	1:9	37.33 42.22	1.13	1:9	22.69 25.39	1.12	1:9	16.90 18.60	1.11
Tetrasulphur[7]helicene (IV)	0:1	5.64 5.82	1.03	0:1	10.37 10.58	1.02	1:9	41.22 43.89	1.06	1:9	21.25 22.10	1.04	1:9	18.40 19.30	1.05
[12]Carbohelicene	1:19	2.94	—	1:19	5.68	—	1:1	4.23 4.85	1.15	1:3	22.36 26.07	1.17	1:1	2.75 3.00	1.09
Dibromo double helicene (I)	1:19	3.80 4.40	1.16	1:19	8.27 9.91	1.20	1:1	7.46 9.62	1.29	1:3	46.86 55.71	1.19	1:1	4.35 5.30	1.22
Dibromo[6]helicene	1:19	3.85 4.08	1.06	1:19	11.09 11.82	1.07	1:1	—**	—	1:3	—**	—	1:1	—**	—
Dibromo[7]helicene	1:19	4.72 4.99	1.06	1:19	11.91 13.19	1.11	1:1	—**	—	1:3	—**	—	1:1	—**	—
Diazadibenzo[7]helicene(II)	1:19	13.60 15.80	1.16	1:9	32.25 40.00	1.24	1:1	63.46 79.85	1.26	1:3	—**	—	1:1	18.05 28.80	1.60

* Mobile phase (m.ph.) in volume ratio of methylene chloride to n-hexane.
** Not eluted.

We have earlier observed with TAPA columns that decreasing the temperature improves resolution[2], and with BPA columns this temperature change led to similar results. Most interesting were the effects on carbohelicene resolutions (Table II). At 2° the former shoulders on the [7]-, [13]- and [14]helicenes had become distinct peaks, although the total resolution (*e.g.*, [7]helicene, $R_s = 0.27$) was still small. In addition, the beginning of resolution had also become visible with the [6]-, [8]- and [9]helicenes.

TABLE II

INFLUENCE OF TEMPERATURE ON CARBOHELICENE RESOLUTION FACTORS USING BPA COLUMNS

The [5]- to [14]carbohelicenes were injected on a 20% linked BPA and a 12% coated BPA column at 2° and 25° using *n*-hexane as mobile phase and a flow-rate of 0.5 ml/min.

Carbohelicene	P(+) BPA linked			P(+) BPA coated		
	$k'_{2°}$	$r_{2°}$	$r_{25°}$	$k'_{2°}$	$r_{2°}$	$r_{25°}$
[5]	2.45	—	—	5.80	—	—
[6]	3.77 3.92	1.04	—	9.36 9.64	1.03	—
[7]	4.79 5.08	1.06	1.04	11.10 11.81	1.06	1.04
[8]	5.38 5.62	1.04	—	12.73 13.25	1.04	—
[9]	7.00 7.23	1.03	—	17.91 18.18	1.02	—
[10]	8.65	—	—	26.55	—	—
[11]	11.00	—	—	40.68	—	—
[12]	14.19	—	—	56.73	—	—
[13]	16.10 16.73	1.04	1.03	64.05 66.14	1.03	1.01
[14]	17.38 18.27	1.05	1.04	69.48 70.76	1.02	1.01

It is interesting that the [7]carbohelicene, in which the terminal benzene rings overlap, was the best resolved on BPA. Resolution was observed to decrease gradually from the [7]- to [9]helicene, was undetectable for the [10]- to [12]helicenes and appeared again after a second helix had been completed ([13]- and [14]helicenes).

This is in contradistinction to TAPA columns, where the [9]helicene was the best resolved of the [5]- to [11]helicenes. Resolution then increased from the [12]- to [14]helicene, with the best resolution for the [14]helicene. Possibly differences in the interplanar angle (*i.e.*, pitch) between a terminal benzene and the overlapping ring, or differences in electron distribution, might determine the variations in helicene resolutions, between one another and towards the two selectors.

Modified selectors

Preliminary tests with nitrated BPA did not lead to the expected improvement in carbohelicene resolutions. While k' values did increase with the stronger CT complexation, the resolution factors were lower (*e.g.*, compound I, $r = 1.05$).

Apparently the nitro groups are a steric hindrance to the selective orientation of the helicenes on the chiral binaphthyl moieties, resulting in loss of chiral recognition.

CONCLUSION

These studies have shown that both complementary topography (chiral recognition) and sufficient binding are required for the resolution of optical isomers by HPLC. Although the topography of the helicenes fits well with both selectors, the stronger binding with TAPA results in higher resolution factors on this selector.

While the total resolution of enantiomers is an important practical aim for the determination of chiroptical properties and biological activities, partial resolutions, when sufficient data are compared, have important theoretical value. (When desired, partial resolutions can always be completed by the use of recycling.)

With the resolution of optical isomers still at an early stage of development, the use of rigid molecules not only helps in predicting and interpreting molecular interactions, but also increases the probability of success. The greater the conformational unambiguity, the higher is the probability of chiral selective interactions.

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