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RESOLUTION OF OPTICAL ISOMERS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY, USING COATED AND BONDED CHIRAL CHARGE-TRANSFER COMPLEXING AGENTS AS STATIONARY PHASES

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SUMMARY

Ten racemic helicenes ([5] to [14]) and two double helicenes {diphenanthro[4,3-*a*; 3′,4′-*o*]picene (I) and 8,20-dibromodiphenanthro[4,3-*a*; 4′,3′-*j*]chrysene (II)} were resolved using high-performance liquid chromatography. R(−)- and S(+)-2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA) and three R(−)-homologues derived from butyric (TABA), isovaleric (TAIVA) and hexanoic (TAHA) acids were used as chiral charge-transfer (C.T.) complex-forming stationary phases, *in situ* coated on silica microparticles. The bulkiness of the group at the chiral centre of the C.T. acceptor is critical for the ease of resolution of the C.T. donors. The [6]–[14]-helicenes and the double helicenes I and II were completely resolved on R(−)-TAPA. On the other hand, the optical isomers of [5]-helicene could be separated only on R(−)-TABA and baseline resolution required ten recycling steps. R(−)-TAPA was also incorporated as a bonded solid phase and as a salt. The resolution factors, *r*, are given, a mechanism of resolution is proposed and the scope of the method and its significance are discussed.

INTRODUCTION

A preliminary communication describing the present work has recently been published[1].

The interaction between chiral selectors and selectands** (solvents and solutes) in gas–liquid chromatography (GLC) has been the subject of investigations in our laboratory for more than a decade[3]. In 1966, the first separation of enantiomers by GLC was reported[4]. Resolution was achieved on capillary columns by selective hydrogen bonding between optically active selectors (stationary phases) and selectands (chiral solutes), both of which were derived from α-amino acids.

With the advent of high-performance liquid chromatography (HPLC), we began, in 1972, to explore the possibility of extending enantiomer separation to this

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** Selector and selectand are cybernetic terms for separating agent and sample input, respectively. They are used to avoid any ambiguities that may arise from the use of the terms solvent–solute, ligand–substrate, host–guest, stationary phase on support or "stationary phase" in mobile phase–sample. The new terms were introduced by one of the authors (F.M.) by analogy with Ashby's operator–operand[2].

method, which is in many aspects more advantageous than GLC. Our approach was based on the assumption that by proper choice of a selector known to exhibit selectivity in its interaction with enantiomers, and its adequate modification if necessary, systems could be found that would lead to efficient resolution under the conditions of HPLC. Many references have appeared in the literature[5–7] on attempts to resolve optical isomers by gravity liquid chromatography (LC). The LC experiments reported in the past either failed to produce satisfactory results, or, where initial successes did arise, were not further pursued. The system that seemed to us to be the most relevant model for our initial investigation was Baczuk *et al.*'s work[8], using low-pressure LC. By linking L-arginine to Sephadex G-25, 3,4-dihydroxyphenylalanine (DOPA) could be resolved after 7 h. Examination of space-filling models showed that the atomic distances and possible contacts, through ionic forces and hydrogen bonds, were such that preferential interaction with D-DOPA would occur.

Our initial studies in HPLC were conducted using covalently linked chiral amino acid or dipeptide moieties, analogous to those used successfully for the resolution of optical isomers by GLC. However, none of our attempts have thus far led to the desired results.

It was considered that the two point hydrogen bonding of diastereomeric associates[3] might be too weak and/or the toposelectivity too limited for the rapid resolution of optical isomers by HPLC. Stronger binding and/or greater spatial recognition appeared to be required. This view was supported by the approach of Cram's group[9–11], where hydrogen and ionic bonding plus inclusion fit permitted strong stereoselective interactions between enantiomers and chiral selectors. By proper design of chiral binaphthyl crown macrocyclic ethers ("hosts"), large differential interactions for chiral "guest" molecules could be produced. This work culminated eventually in the development of columns with bonded chiral selectors[10,11], permitting the resolution of amino acid esters and chiral aromatic amines by HPLC.

In parallel with the above work, we had initiated the study of resolution with the help of chiral charge-transfer (C.T.) selectors. It is well known that high selectivities can be achieved by complexation[12–14]. Before tackling the problem of enantiomeric separation, preliminary experiments were conducted in order to check the performance of complex-forming selectors in HPLC. In 1973, we reported[15] that Corasil II coated with silver nitrate in ethylene glycol or rhodium(II) tetraacetate gave good separation of olefins, which emerged from the column in the order of their respective stability constants. Additional publications[16–18] on the HPLC separation of aromatic hydrocarbons using achiral C.T. selectors have since appeared.

A chiral C.T. complexing agent, 2-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA), had been introduced by Newman and Lednicer[19] for the separation of hexahelicene*[20] enantiomers by crystallization, and had been used with a certain measure of success for the resolution of other optically active aromatic compounds[21,22]. Our above preliminary experiments and the limited degree of LC resolution obtained with TAPA reported in the literature[21–24] led to the conclusion that the main experimental effort would have to be concentrated on increasing column

* The nomenclature of helicenes is established by the "helicity rule" (IUPAC rules A-21, A-22, B-3 and B-4). Depending on whether the identified helix is left- or right-handed, it is designated "minus" and denoted by M, or designated "plus" and denoted by P. The prefixes (+) and (—) indicate the sense of the optical rotation of the compound.

efficiency. The procedure that was eventually worked out in order to obtain a high number of plates* is described under Experimental.

Experiments with various aromatic compounds containing chiral aliphatic substituents (C.T. donors) indicated that even high-efficiency chiral columns were not necessarily adequate for the resolution of optical isomers. It was postulated that interpenetration of one molecule into a cavity of the other might be required. We therefore chose to concentrate on the helicenes as C.T. donors, as they possess semi-cavities at both ends of their helical structure. Examination of space-filling models showed the good preferential fit of one helicene enantiomer over the asymmetric centre of TAPA.

The helicenes[20,25], composed of *o*-condensed benzene rings, are inherently chiral and palindromic, belonging to point group C_2. They possess the highest optical rotations yet reported for organic molecules {*e.g.*, [9]-helicene, $[\alpha]_{579}^{25} = 8100°$ (ref. 26)}. In spite of the initial success with the [6]-helicene[19], Wynberg in a recent review[27] states that "the resolution of helicenes has proved both exciting and frustrating". Indeed, the crystallization and chromatographic techniques used in the last 20 years were found to be time consuming and often led to incomplete separation[21–24,28,29]. The development of efficient and rapid new procedures of resolution appeared, therefore, to correspond to an urgent need in this area of research.

EXPERIMENTAL

Apparatus

A self-constructed HPLC system was equipped with a reciprocating two-piston delivery system (Model M 6000, Waters Assoc., Milford, Mass., U.S.A.). Seamless type 316 stainless-steel tubing 0.05 cm I.D. (Superior Tube Co., Norristown, Pa., U.S.A.) was used for all connections. Two injectors of similar type were used: one, a modified version of that described by Halász *et al.*[30], was constructed in our workshop; the second was a septum injector kit (Type C904, Waters Assoc.). Columns were prepared from stainless-steel tubing, 0.32 cm O.D. × 0.23 cm I.D. (Analabs Inc., North Haven, Conn., U.S.A.), fitted with Swagelok reducing unions (Type 200-6-1, Crawford Fitting Co., Cleveland, Ohio, U.S.A.) drilled out to minimize the dead volume[31]. A stainless-steel porous disc with 5-μm pores, 0.3 cm diameter × 0.16 cm thick (Mott Metallurgical Corp., Framington, Conn., U.S.A.) was placed at the bottom of each column inside the reducing union. An ultraviolet detector operating at 254 or 280 nm (Model 1205, Laboratory Data Control, Riviera Beach, Fla., U.S.A.) was used.

The stainless-steel columns (20 cm × 0.23 cm I.D.) were packed by the high-viscosity slurry technique[32,33], using either carbon tetrachloride or tetrabromoethane–tetrachloroethylene to suspend the microporous silica particles. The support materials used were Partisil 7 (7 μm average particle size), Partisil 10 (10 μm) (Reeve Angel Scientific, London, Great Britain) and LiChrosorb SI 100 (5 μm) (E. Merck, Darmstadt, G.F.R.). The microparticles (0.8 g) were suspended in 10 ml of carbon tetrachloride or tetrabromoethane–tetrachloroethylene (3:2, w/w), ultrasonically degassed (Ultrasonic Vibrator, Varian, Palo Alto, Calif., U.S.A.) and introduced into a filling reservoir with 35 ml capacity (modified Yeda Press, Yeda Research and Development Co. Ltd., Weizmann Institute of Science, Rehovot, Israel) above the

* Comparable with those obtained on the best commercial columns (*e.g.*, 25,000 plates per metre).

column. The reservoir was carefully filled to the top with carbon tetrachloride and pressurized to 6000 p.s.i. with heptane as the mobile phase. The filling time was 20–30 min. A freshly prepared column gave about 5000 theoretical plates per 20-cm column [u (= linear velocity = length of column (cm)/dead volume (sec)) = 0.20] for naphthalene on Partisil 7, 1600 on Partisil 10, and 1800 on LiChrosorb SI 100, using hexane as the mobile phase.

The instruments used for the analysis of selectors and enantiomers included a laser ultramicropolarimeter (Type UM 141, Perkin Elmer, Norwalk, Conn., U.S.A.) provided with a helium–neon laser source (Model 133, Spectra Physics, Santa Clara, Calif., U.S.A.), a Cary 14 recording UV spectrophotometer and a Cary 14 provided with circular dichroism accessory 6002 (Applied Physics Corp., Monrovia, Calif., U.S.A.), a 21-490B single-focusing mass spectrometer (DuPont, Wilmington, Del., U.S.A.), a quadrupole mass spectrometer system 1015 GC/MS (Finnigan Instruments Corp., Palo Alto, Calif., U.S.A.), the former for helicene analysis and the latter for the selectors, using direct inlet probes, and a basic system T60 nuclear magnetic resonance spectrometer (Varian).

Chemicals

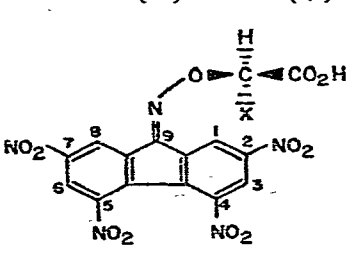
The solvents used were either spectroscopic grade (Fluka, Buchs, Switzerland) or chemically pure (Frutarom, Haifa, Israel). The latter were further purified by treatment with 5% potassium permanganate solution, then with 5% sulphuric acid, followed by chromatography on silica gel and alumina (Merck), and distilled.

Materials used for the synthesis of the asymmetric selectors included 2,4,5,7-tetranitrofluorenone, (—)-ephedrine and N,N′-dicyclohexylcarbodiimide (DCC) (Fluka), ethyl 2-bromopropionate and ethyl 2-bromobutyrate (BDH, Poole, Great Britain), ethyl 2-bromo-3-methylbutyrate and 2-bromohexanoic acid (Eastman Organic Chemicals, Rochester, N.Y., U.S.A.) and 3-aminopropyltriethoxysilane (Union Carbide, New York, N.Y., U.S.A.).

The helicenes were generously provided by various laboratories: [5]- and [7]–[14]-helicenes and the two double helicenes diphenanthro[4,3-*a*; 3′,4′-*o*]picene (I) and 8,20-dibromodisphenanthro[4,3-*a*; 4′,3′-*j*]chrysene (II) by Prof. R. H. Martin, Brussels, [6]-helicene by Prof. H. A. Staab, Heidelberg, and [8]-helicene by Prof. H. Kagan, Paris.

Synthesis of chiral selectors

Chiral R(—)- and S(+)-TAPA and the enantiomeric homologues TABA,



X = methyl, R(—)-TAPA
X = ethyl, R(—)-TABA
X = isopropyl, R(—)-TAIVA
X = butyl, R(—)-TAHA

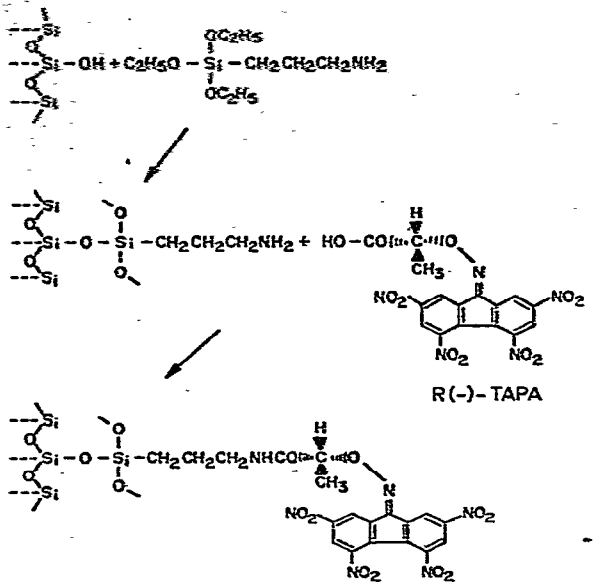


Fig. 1. Synthesis of bonded chiral selector.

TAIVA and TAHA were synthesized according to Block and Newman[34].

Both enantiomers of each acid were prepared and identified by microanalysis, mass spectrometry, nuclear magnetic resonance spectrometry, polarimetry and melting point determinations (Table I).

Chiral bonded selector

The covalent linking was carried out in two steps (Fig. 1). 3-Aminopropyltriethoxysilane (1.3 mmole) was first coupled with 1 g of microsilica particles (Partisil 7) by suspending the particles with stirring in a 30% solution of the amine coupling reagent in toluene, and then refluxing at 120° for 8 h. The aminated support was washed with toluene, acetone and methanol and dried for 2 h under vacuum at 110°.

The asymmetric moiety, R(—)-TAPA, was then covalently linked to the aminated silica particles using DCC as coupling reagent in dry chloroform. The reaction was carried out at room temperature with stirring for 4 h. The bonded chiral selector was exhaustively washed with chloroform, acetone and methanol, then slurry-packed into a 20 × 0.23 cm I.D. column as previously described.

In situ coating of chiral selectors

Columns coated with R(—)- and S(+)-TAPA, R(—)-TABA, R(—)-TAIVA and R(—)-TAHA were prepared by the *in situ* technique[35]. A saturated solution of one enantiomer in nitromethane or dioxan was injected repetitively on to the column, using hexane saturated with nitromethane or dioxan as the mobile phase at a flow-rate of 0.2 ml/min. The percentage of coating applied for each chiral stationary phase is given in Table I.

A column with R(—)-TAPA coated as a salt was prepared in a similar way. The chiral selector was injected on to a column filled with 3-aminopropylsilanized microsilica particles.

TABLE I

CHARACTERIZATION OF CHIRAL COATED AND BONDED SELECTORS

Selector	$[\alpha]_{633}^{23}$*	M^+-CO_2H	*Elemental analysis (%)*		*Melting point (°C)*	*Column characteristics*				
			Found C H	*Theor.* C H		*Support*	*Mode of application*	*Dimensions (cm)*	*Selector (%)*	*N/m***
R(−)-TAPA	− 90*****	402	43.01 2.02	42.96 2.03	200–201	Partisil 7	coated salt bonded	20 × 0.23 20 × 0.23 20 × 0.23	25 13 18	14,000 3000 3000
S(+)-TAPA	+ 85§	402	43.09 2.08	42.96 2.03	196–198	Partisil 7	coated	2 × 20 × 0.23†	11	12,000
R(−)-TABA	− 88	416	44.23 2.38	44.26 2.40	184–185	Partisil 7	coated	2 × 20 × 0.23†	20	8000
S(+)-TABA	+ 83§	416	—	—	183–185	—	—	—	—	—
R(−)-TAIVA	−103	430	45.00 2.90	45.48 2.76	200–202	Partisil 10§§§	coated	20 × 0.23	15	1800
R(−)-TAHA	− 22§§	444	47.21 3.66	46.63 3.09	207–209	LiChrosorb SI 100§§§	coated	20 × 0.23	13	1700

* (*c* 1.5, $CHCl_3$), measured in a 100-μl microcuvette.

** Plates per metre measured for naphthalene, $u = 0.25$ cm/sec, mobile phase hexane.

*** $[\alpha]_{589}^{25} = -97°$ (ref. 34).

§ [α] value is lower as the (+)-enantiomer was recovered from the mother liquor.

§§ Value possibly too low (see text).

§§§ Partisil 10 and LiChrosorb SI 100 gave less efficient columns than Partisil 7, which was not available during the latter part of this work.

† Two 20 × 0.23 cm columns, coupled by a Swage lok dead volume union, $^1/_8 \times {}^1/_8$ in.

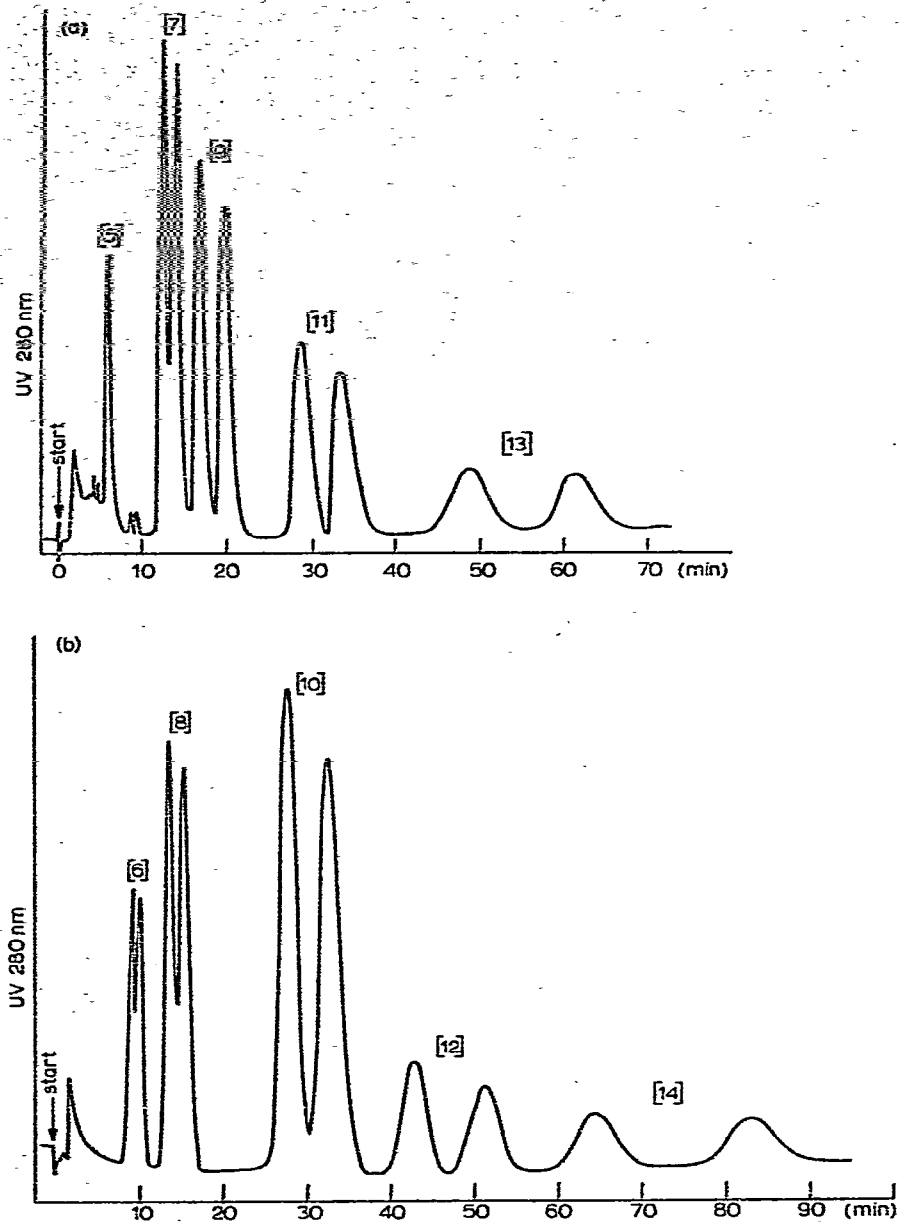


Fig. 2. Resolution of carbohelicenes: (a) mixture of the racemates of [5]-, [7]-, [9]-, [11]- and [13]-helicenes and (b) of [6]-, [8]-, [10]-, [12]- and [14]-helicenes. The more strongly retained enantiomer was the P(+)-helicene in all instances. Column: 25% R(−)-TAPA, mobile phase 25% dichloromethane–cyclohexane, $u = 0.26$ cm/sec.

TABLE II

RESOLUTION OF HELICENES BY HPLC USING CHIRAL TAPA HOMOLOGUES AS SELECTORS

[5]-[14]-Helicenes and double helicenes I and II were separated on four chiral columns under the following conditions: (1) R(−)-TAPA, 25% coated on Partisil 7, 20 × 0.23 cm, u = 0.26 cm/sec.; (2) R(−)-TABA, 20% coated on Partisil 7, 2 × 20 × 0.23 cm, u = 0.24 cm/sec.; (3) R(−)-TAIVA, 15% coated on Partisil 10, 20 × 0.23 cm, u = 0.26 cm/sec.; (4) R(−)-TAPA, 18% bonded on 3-aminopropyl-silanized Partisil 7, 20 × 0.23 cm, u = 0.28 cm/sec. The mobile phase in all instances was 25% dichloromethane-cyclohexane and the temperature was 23–25°.

Helicene	*R(−)-TAPA (coated)*				*R(−)-TABA (coated)*				*R(−)-TAIVA (coated)**			*R(−)-TAPA (covalently bonded)*			
	k'	k'/k' [5]	r	R_s	k'	k'/k' [5]	r	R_s	k'	k'/k' [5]	r	k'	k'/k' [5]	r	R_s
[5]	2.68	1.00	—	—	3.91	1.00	1.033	0.33	1.73	1.00	—	2.65	1.00	1.049	0.18
					4.04	1.03						2.78	1.05		
[6]	4.08	1.52	1.113	0.75	5.87	1.50	1.112	0.99	2.50	1.45	1.048	3.87	1.46	1.124	0.40
	4.54	1.69			6.53	1.67			2.62	1.51		4.35	1.64		
[7]	6.15	2.29	1.138	0.96	8.75	2.24	1.104	1.00	3.54	2.05	1.023	5.70	2.15	1.167	0.64
	7.00	2.61			9.66	2.47			3.62	2.09		6.65	2.51		
[8]	6.58	2.46	1.147	1.04	8.78	2.25	1.093	0.91	3.42	1.98	1.024	5.61	2.12	1.171	0.63
	7.55	2.82			9.60	2.46			3.50	2.02		6.57	2.48		
[9]	8.57	3.20	1.187	1.48	11.76	3.01	1.131	1.31	4.39	2.53	1.034	7.04	2.66	1.179	0.61
	10.17	3.79			13.31	3.40			4.54	2.62		8.30	3.13		
[10]	12.81	4.78	1.162	1.35	18.15	4.64	1.092	0.93	6.19	3.58	1.019	10.26	3.87	1.140	0.52
	14.89	5.56			19.82	5.07			6.31	3.65		11.70	4.41		
[11]	13.67	5.10	1.156	1.40	21.86	5.59	1.069	0.65	7.19	4.16	1.028	11.48	4.33	1.147	0.50
	15.80	5.90			23.36	5.97			7.39	4.27		13.17	4.97		
[12]	17.53	6.54	1.200	1.55	25.89	6.62	1.101	0.87	8.69	5.02	1.053	13.44	5.07	1.181	0.71
	21.04	7.85			28.50	7.24			9.15	5.29		15.87	5.99		
[13]	21.44	8.00	1.234	1.81	32.61	8.34	1.108	0.97	10.62	6.14	1.057	15.96	6.03	1.190	0.58
	26.45	9.87			36.14	9.24			11.23	6.49		19.00	7.17		
[14]	23.58	8.80	1.277	2.18	39.29	10.05	1.153	1.39	10.92	6.31	1.064	18.74	7.07	1.218	0.61
	30.12	11.24			45.29	11.58			11.62	6.72		22.83	8.61		
I**	16.24	6.06	1.193	1.53	—	—	—	—	—	—	—	12.30	4.64	1.144	0.48
	19.38	7.23										14.07	5.31		
II**	30.42	11.35	1.236	1.68	—	—	—	—	—	—	—	23.27	8.78	1.273	0.92
	42.32	14.05										29.62	11.18		

* Resolution was small with R_s <0.30, but shoulders could be definitely detected in all instances.

** Double helicenes; see Fig. 8 for structures.

RESULTS AND DISCUSSION

[6]–[14]-Helicenes

Hexahelicene, on a column coated with R(—)-TAPA, was the first racemate that we succeeded in resolving by HPLC ($r^* = 1.112$), producing a chromatogram with two well separated peaks of equal area. The first confirmation of actual enantiomer separation was obtained by isolating small amounts of material and determining the sign of rotation [1st peak (—); 2nd peak (+)]. On S(+)-TAPA these samples emerged in the opposite order to that on the first column, and the (—) compound showed spiking of the second peak.

The [7]–[14]-helicenes were then resolved under similar conditions, as can be seen in Fig. 2. The value of the resolution factor (r) and resolution function (R_s) of each compound are listed in Table II. At room temperature, [6]–[10]-helicenes were incompletely resolved ($R_s < 1.25$). However, by operating at lower temperatures, baseline separation could be achieved. The influence of temperature on r values for [6]-helicene is illustrated in Fig. 3. The broadening of the peaks at lower temperatures is more than compensated for by the increase in the r values.

Once the conditions for the resolution of the various helicenes had been optimized, final confirmation of the nature of the enantiomeric peaks was produced by the isolation of 8–12-μg samples. The specific optical rotations (reported in a preliminary communication[1]) were found to be in agreement with literature data where available, and the ultraviolet and mass spectra were in accord with the structure. Furthermore, the absolute configuration, (M), of the [9]–[14]-(—)-enantiomers, *i.e.*, those which are less strongly retained on R(—)-TAPA, was determined by circular dichroism. The Cotton effect was indeed found to be of the same sign [*e.g.*, (—)-[9]-helicene, $[\theta]^{23}_{389} = -0.96 \cdot 10^5°$] as has been recorded in the literature for the M(—)-[5]- to M(—)-[9]-helicenes[36–38] between 300 and 400 nm.

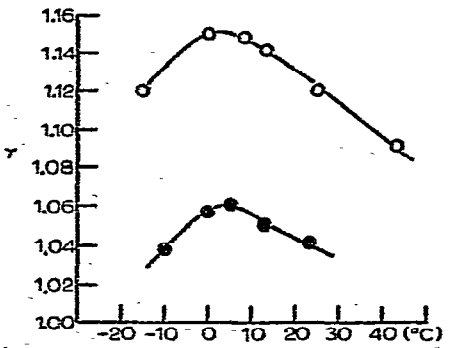


Fig. 3. Influence of temperature on resolution factors. ○, [6]-Helicene on R(—)-TAPA between —15 and 45°; ●, [5]-helicene on R(—)-TABA between —10 and 25°.

* r, the resolution factor (= retention volume of less mobile enantiomer minus dead volume/retention volume of more mobile enantiomer minus dead volume). This parameter is generally designated by α, the separation factor, but as this symbol is also used for optical rotation its use in the present context could be confusing.

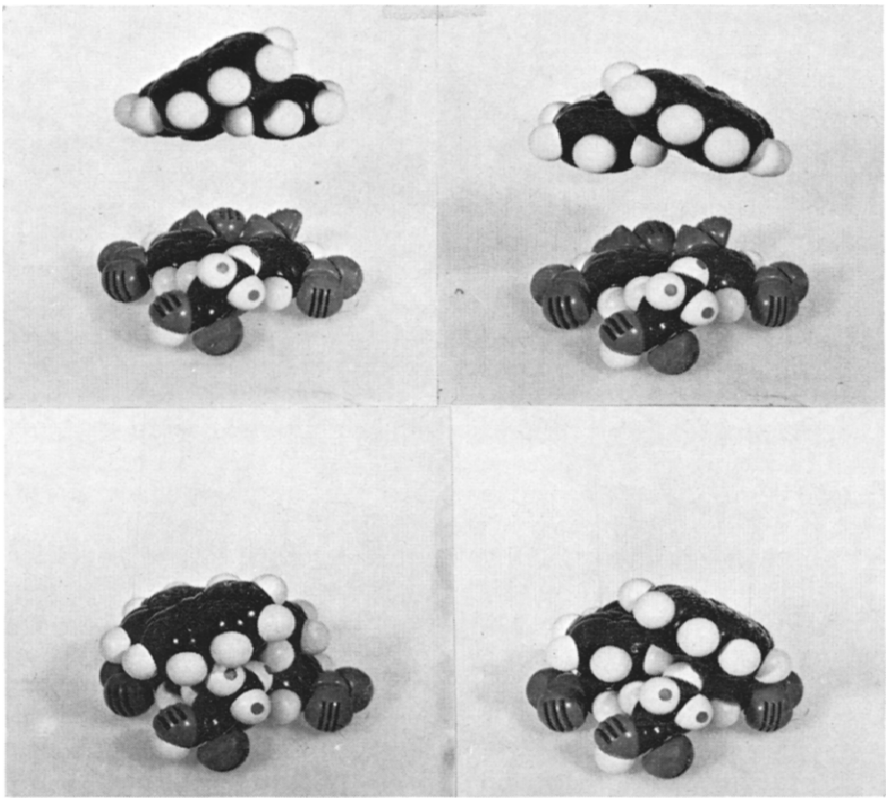


Fig. 4. CPK models of diastereomeric associates. Left-hand side: P(+)-[6]-helicene, R(−)-TAPA and complex (more favoured). Right-hand side: M(−)-[6]helicene, R(−)-TAPA and complex (less favoured). Hydrogens of the methyl groups are marked with black spots.

The preferential complexation of R(−)-TAPA with the P(+)-[6]-helicene enantiomer can be seen with the spacefilling (CPK; Corey–Pauling–Kolton) models in Fig. 4, where the semi-cavity of the latter fits comfortably over the corresponding "cone" formed by the H and CH_3 groups at the asymmetric carbon of TAPA.

[5]-Helicene

As shown in Fig. 5, overlap of the terminal rings in [5]-helicene is restricted to the hydrogen atoms. Also, the pitch of the helix is smaller than in the higher analogues and racemization is known to occur readily at room temperature. It is, therefore, not surprising that [5]-helicene was particularly difficult to resolve. On a TAPA-coated column, no separation of enantiomers could be observed (Fig. 6a). However, when the alkyl group at the asymmetric carbon of the C.T. acceptor was increased to ethyl, a partial separation resulted (Fig. 6b), and baseline resolution could be achieved after ten recycling steps at 0° on two coupled columns, each 20 × 0.23 cm (Fig. 7). Identification of the peaks was carried out as above.

Temperature studies with [5]-helicene on R(−)-TAPA (Fig. 3) indicated a maximum r value between 0 and 8°, as for [6]-helicene (see above). The reason for

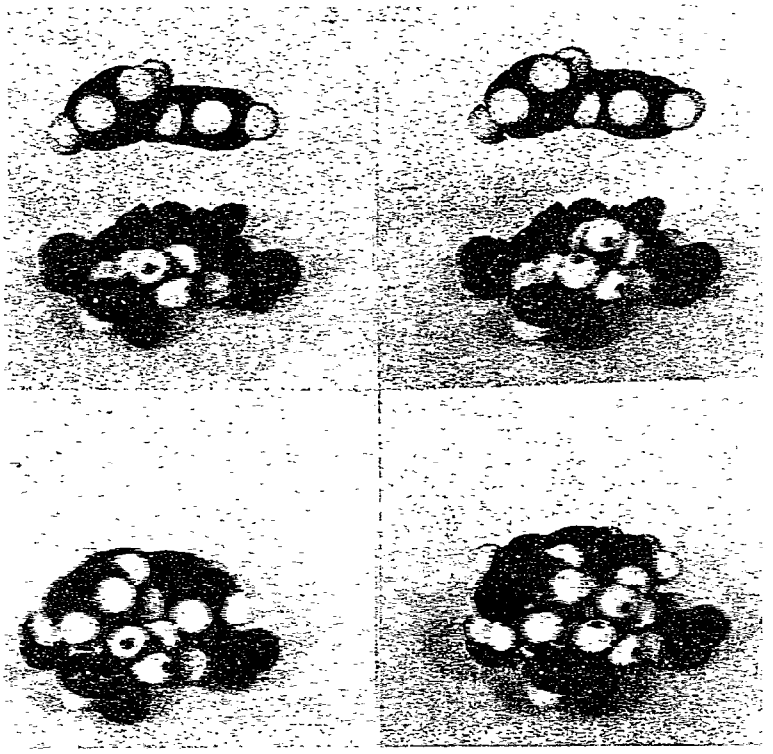


Fig. 5. CPK models of diastereomeric associates. Left-hand side: P(+)-[5]-helicene, R(−)-TAPA and the complex (less selective). Right-hand side: P(+)-[5]-helicene, R(−)-TABA and the complex (more selective). Hydrogens of the methyl and ethyl groups are marked with black spots.

this phenomenon has not yet been elucidated and we are at present investigating its solvent dependence.

The r and R_s values for the [6]–[14]-helicenes on R(−)-TABA were also measured (Table II). Except for [6]-helicene, they were lower than those on TAPA in all instances.

Double helicenes

The double helicenes I and II (for structural formulae and chromatogram, see Fig. 8) were readily resolved on S(+)- and R(−)-TAPA-coated columns. Compound I, which can be considered as a double hexahelicene hinged on a benzene unit (total number of rings 11), has about the same retention and r value as [12]-helicene (Table II). Compound II is also a double hexahelicene, but is hinged on a naphthalene unit (total number of rings 10), and contains a bromine atom in each helix. Its retention is greater than that of [14]-helicene and the r value is about the same as for [13]-helicene (Table II). This behaviour can no doubt be ascribed to the increase in the electron donating capacity of the selectand following bromine substitution.

Selector covalently bonded to support

The above compounds can be resolved equally well on TAPA covalently

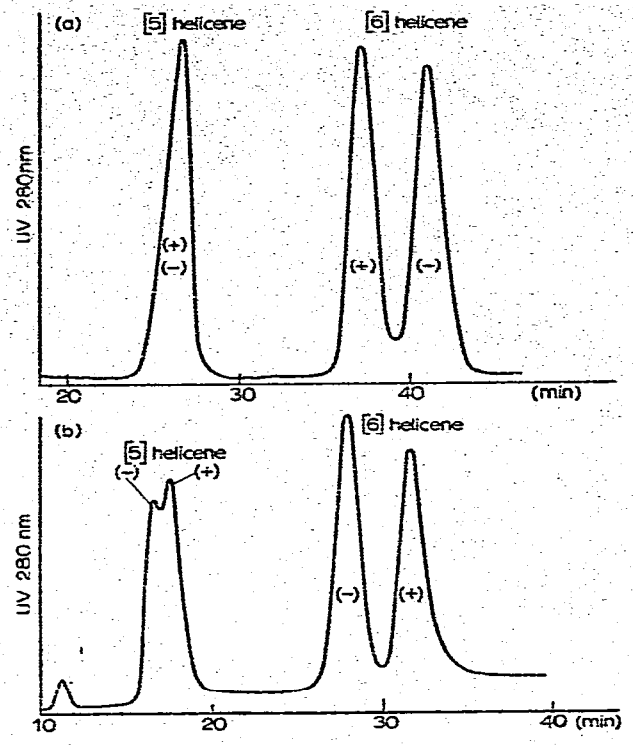


Fig. 6. Chromatogram of a mixture of racemic [5]- and [6]-helicenes on S(+)-TAPA and R(−)-TABA. (a) Column: 11% S(+)-TAPA–Partisil 7, 2 × 20 × 0.23 cm I.D., mobile phase 20% dichloromethane–cyclohexane, $u = 0.20$ cm/sec, 25°. [5]-Helicene is not resolved. (b) Column: 20% R(−)-TABA–Partisil 7, 2 × 20 × 0.23 cm I.D., mobile phase 25% dichloromethane–cyclohexane, $u = 0.24$ cm/sec, 25°.

linked to Partisil 7 (Table I). The r values for [6]-, [7]- and [8]-helicene were found to be even higher than on coated columns and a small resolution effect for [5]-helicene was observed (Table II and Fig. 9). On the other hand, the resolution functions (R_s) were in all instances lower than on coated columns. A 3-aminopropyl-silanized Partisil 7 column on which R(−)-TAPA was linked ionically as a salt (Table I) gave even

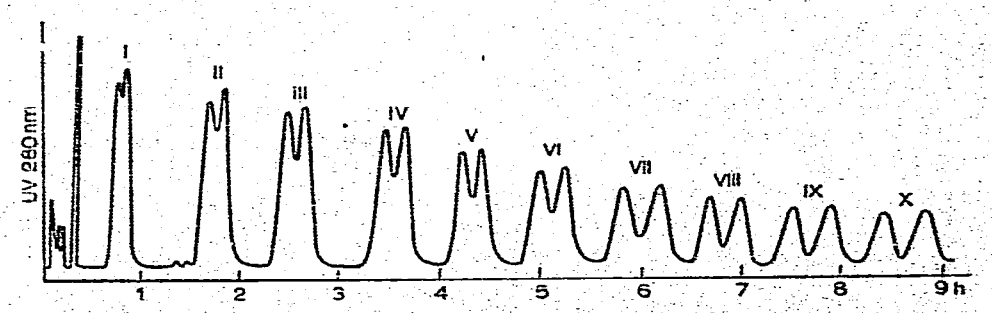


Fig. 7. Resolution of [5]-helicene by recycling on R(−)-TABA. $u = 0.17$ cm/sec, 0°; other chromatographic conditions as in Fig. 6b.

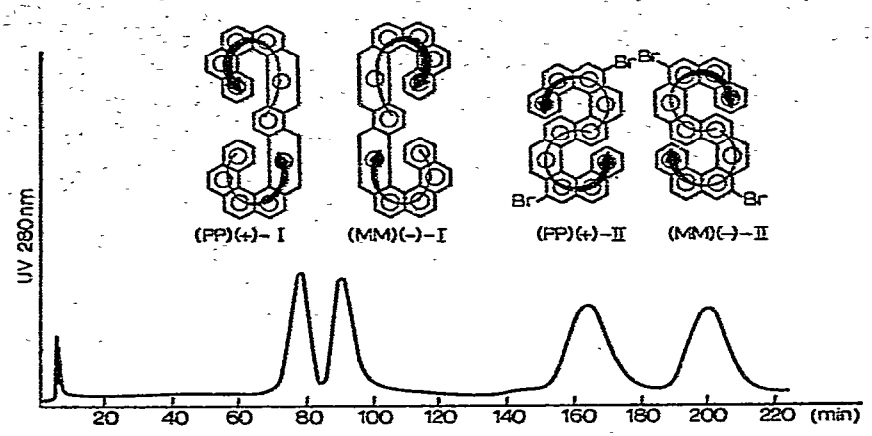


Fig. 8. Resolution of the racemic double helicenes I and II. Column: 11% S(+)-TAPA, mobile phase 25% dichloromethane–cyclohexane, $u = 0.23$ cm/sec. The arrows in the formulae point in the sense of the ascending helical turns.

lower R_s values. Modified supports never pack as efficiently as untreated supports (see Table I for plate numbers), and they present higher resistance to mass transfer. However, the demonstration that linked chiral selectors show selectivity for enantiomer separations has great interest, as they permit the use of a wide range of mobile phases, particularly of a polar nature. The further development of covalently linked TAPA columns is now in progress, and it appears likely that columns equal in efficiency to the coated ones could be produced.

Structural modification of chiral selectors

In addition to TAPA and TABA, two other homologues (Table I) of the selector were prepared from α-bromoisovaleric (TAIVA) and α-bromo-*n*-hexanoic acid (TAHA) for the purpose of studying further the influence on resolution of ligand size at the asymmetric carbon. Small resolution effects were observed on a column

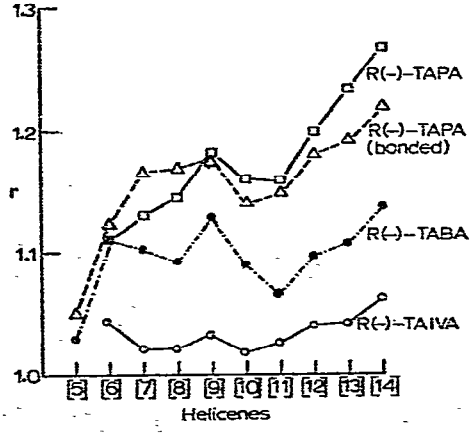


Fig. 9. Resolution factors for helicenes on different chiral selectors. Chromatographic conditions are given in Table II.

coated with TAIVA for the [6]–[14]-helicenes, but none for the [5]-helicene. For TAHA, no distinct resolution could be detected, although the column used had the same efficiency as that containing TAIVA. It should be mentioned, however, that the low specific rotation of TAHA, in spite of several recrystallizations of its ephedrine salt (Table I), casts some doubt on the optical purity of this selector. A quantitative method for the determination of chiral selector optical purity is now being sought.

A comparison of the r values for the [5]–[14]-helicenes on coated TAPA, TABA, TAIVA and bonded TAPA columns is given in Fig. 9. The r values on all columns can be seen, in general, to increase with the number of rings in the molecule of the selectand with a discontinuous growth for the [9]-helicene. The r values, however, have not yet been corrected for the contribution made by the non-selective interaction of both the siliceous support and the achiral moiety of the selector. It would therefore be premature to attempt a discussion of the relationship between selectand structure and selectivity at this time.

Selector–selectand interaction

Some conclusions can be drawn concerning the molecular reasons for the selectivity of the selector–selectand interaction. Klemm *et al.*[39] and Haenel and Staab[24,29] assumed that the TAPA acid hydrogen is internally chelated with the imino nitrogen, but differ in the orientation assumed for the methyl group with respect to the plane passing through the fluorenylidene moiety. We feel that internal hydrogen bonding is not an essential factor, as high r values were obtained on columns containing linked TAPA, where chelation would appear not to occur*.

In Fig. 10, we have assumed that the selector molecules are oriented parallel to the surface of the support with the methyl group as far as possible turned away from it**. Looking at these structures, it can be seen that the semi-cavity of the P(+)-selectand will readily enclose the hydrogen and methyl group attached to the asymmetric carbon of the R(−)-selector. On the other hand, these substituents of the asymmetric carbon tend to lift the M(−)-selectand off the surface of the selector, thus diminishing the overlap of the π-electrons.

A larger alkyl group at the asymmetric carbon will not be readily accomodated by the semi-cavities of [6]–[14]-helicenes, all of which should have essentially the same shape. Thus, the decrease in the r values with increasing size of the alkyl substituents can readily be understood.

Pentahelicene, on the other hand, has a semi-cavity of a different shape than the higher analogues (Fig. 5), as already pointed out above. Correspondingly, the effect of the alkyl substituent of the selector is different. Separation can hardly be observed for the methyl substituent (TAPA), whereas resolution was achieved with an ethyl group (TABA). For larger alkyl groups (TAIVA and TAHA), the resolution dropped to zero.

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* For the covalently linked TAPA, the possible NH···N bond is not only inherently weak, but also sterically hindered; on the TAPA salt column (r = 1.25 for hexahelicene), the acid hydrogen is transferred to the amino group.

** The similarity of the profiles representing the selector molecule with that given by Klemm *et al.*[39] should not be construed to mean that we use a chelated structure for our model.

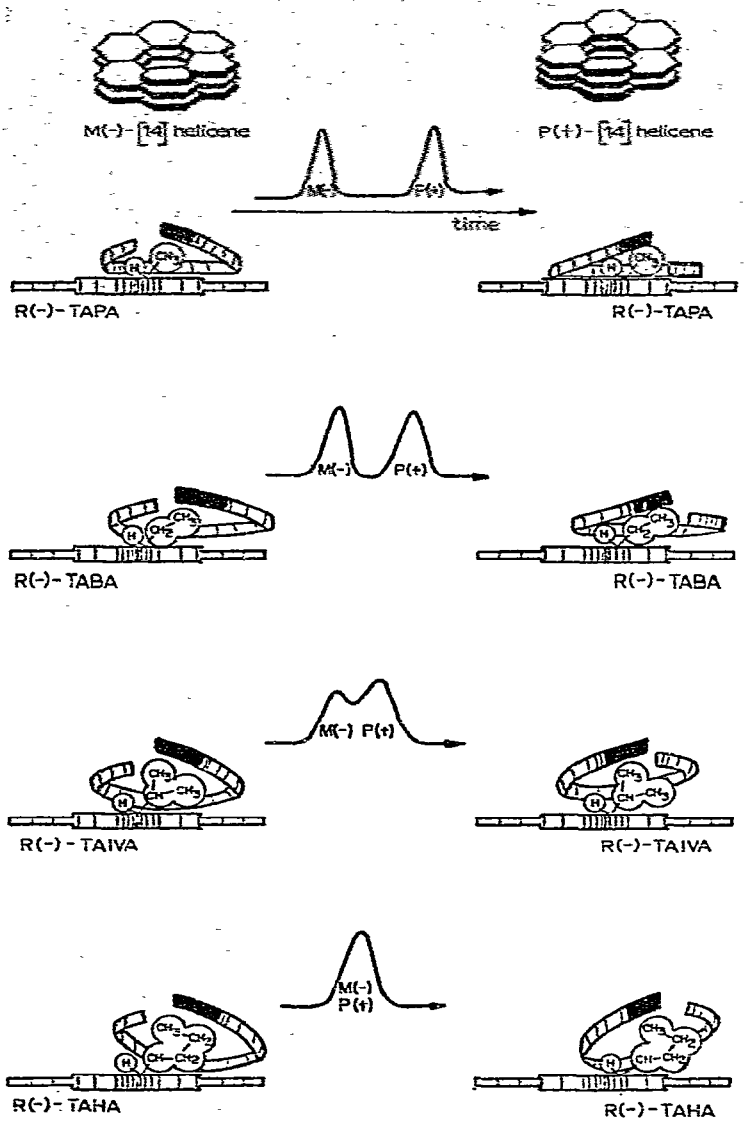

Fig. 10. Suggested explanation for the gradual decrease in resolution with increasing size of the ligands at the asymmetric carbon of TAPA homologues. The chromatograms shown are for the best resolved compound ([14]-helicene).

In addition to the influence of the size and shape of the selectand semi-cavity, the C.T. capacity of the selectand is an essential factor. This is strikingly demonstrated, as mentioned above, by the bromo-substituted double helicene II. The conclusions presented above were based largely on a consideration of CPK models. For a deeper understanding of the selectivity of the system, X-ray crystallographic studies of the helicenes and diastereomeric selector–selectand complexes are required. To date, only the X-ray studies on [6]-helicene[40] and the C.T. complex of [6]-helicene with

achiral 4-bromo-2,5,7-trinitrofluorenone[41] have been reported, although others are in progress[42,43].

CONCLUSION

This procedure should be of considerable interest for the study of helicene chiro-optical properties and such chemical reactions as thermal, and possibly photochemical, racemization and asymmetic inductions. This approach should also be extendable to other classes of selectands that are capable of forming C.T. complexes or that have functions through which suitable aromatic moieties could be introduced. It should also be possible to reverse the functions of the selectors and selectands described here.

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REFERENCES

1 F. Mikeš, G. Boshart and E. Gil-Av, *Chem. Commun.*, (1976) 99.
2 W. R. Ashby, *An Introduction to Cybernetics*, Chapman & Hall, London, 1956, pp. 10–16 and 259–264.
3 E. Gil-Av, *J. Mol. Evol.*, 6 (1975) 131.
4 E. Gil-Av, B. Feibush and R. Charles-Sigler, in A. B. Littlewood (Editor), *Gas Chromatography 1966*, Institute of Petroleum, London, 1967, p. 227.
5 D. R. Buss and T. Vermeulen, *Ind. Eng. Chem.*, 60 (1968) 12.
6 P. H. Boyle, *Quart. Rev., Chem. Soc.*, (1971) 323.
7 S. H. Willen, *Tables of Resolving Agents and Optical Resolution*, University of Notre Dame, Notre Dame Press, Indiana, 1972.
8 R. J. Baczuk, G. K. Landrum, R. J. Dubois and H. C. Dehm, *J. Chromatogr.*, 60 (1971) 351.
9 L. R. Sousa, D. H. Hoffman, L. Kaplan and D. J. Cram, *J. Amer. Chem. Soc.*, 96 (1974) 7100.
10 G. D. Y. Sogah and D. J. Cram, *J. Amer. Chem. Soc.*, 97 (1975) 1259.
11 G. D. Y. Sogah and D. J. Cram, *J. Amer. Chem. Soc.*, 98 (1976) in press.
12 O. K. Guha and J. Janák, *J. Chromatogr.*, 68 (1972) 325.
13 R. Foster, *Organic Charge-Transfer Complexes*, Academic Press, New York, 1969.
14 R. Foster (Editor), *Molecular Association*, Academic Press, New York, 1975.
15 F. Mikeš, V. Schurig and E. Gil-Av, *J. Chromatogr.*, 83 (1973) 91.
16 R. L. Karger, M. Martin, J. Loheac and G. Guichon, *Anal. Chem.*, 45 (1973) 496.
17 C. H. Lochmüller and C. W. Amoss, *J. Chromatogr.*, 108 (1975) 85.
18 G. Schomburg and K. Zegarski, *J. Chromatogr.*, 114 (1975) 174.
19 M. S. Newman and D. Lednicer, *J. Amer. Chem. Soc.*, 78 (1956) 4765.
20 M. S. Newman, W. B. Lutz and D. Lednicer, *J. Amer. Chem. Soc.*, 77 (1955) 3420.
21 L. H. Klemm and D. Reed, *J. Chromatogr.*, 3 (1960) 364.
22 L. H. Klemm, K. B. Desai and J. R. Spooner, Jr., *J. Chromatogr.*, 14 (1964) 300.
23 W. Rebafka and H. A. Staab, *Angew. Chem., Int. Ed. Engl.*, 12 (1973) 776.
24 M. Haenel and H. A. Staab, *Chem. Ber.*, 106 (1973) 2203.
25 R. H. Martin, *Angew. Chem., Int. Ed. Engl.*, 13 (1974) 649.
26 R. H. Martin and M. J. Marchant, *Tetrahedron*, 30 (1974) 343.
27 H. Wynberg, *Acc. Chem. Res.*, 4 (1971) 65.
28 H. Rau and O. Schuster, *Angew. Chem.*, 88 (1976) 90.
29 M. Haenel, personal communication.

30 I. Halász, A. Kroneissen, H. O. Gerlach and P. Walking, *Z. Anal. Chem.*, 234 (1968) 88.
31 F. M. Rabel, *Int. Lab.*, July/August (1975) 38.
32 B. Coq, C. Gonnet and J. L. Rocca, *J. Chromatogr.*, 106 (1975) 249.
33 R. M. Cassidy, P. S. le Gay and R. W. Frei, *Anal. Chem.*, 46 (1974) 340.
34 P. Block and M. S. Newman, *Org. Syn.*, 48 (1968) 120.
35 J. J. Kirkland and C. D. Dilks, Jr., *Anal. Chem.*, 45 (1973) 1778.
36 C. Goedicke and H. Stegemeyer, *Tetrahedron Lett.*, (1970) 937.
37 J. Tribout, R. H. Martin, M. Doyle and H. Wynberg, *Tetrahedron Lett.*, (1972) 2839.
38 R. H. Martin and M. J. Marchant, *Tetrahedron*, 30 (1974) 343.
39 L. H. Klemm, W. Stalick and D. Bradway, *Tetrahedron*, 20 (1964) 1667.
40 C. de Rango, G. Tsoucaris, J. P. Declerq, G. Germain and J. P. Putzeys, *Cryst. Struct. Commun.*, 2 (1973) 189.
41 I. R. Mackay, J. L. Robertson and J. G. Sime, *Chem. Commun.*, (1969) 1470.
42 G. W. Frank, J. Jespers and R. H. Martin, unpublished results.
43 G. Tsoucaris, personal communication.