01 Oct 1988

Enantiomeric Resolution And Chiral Recognition Of Racemic Nicotine And Nicotine Analogues By **B**-cyclodextrin Complexation: Structure-enantiomeric Resolution Relationships In Host-guest Interactions

Jeffrey I. Seeman

Henry V. Secor

Daniel W. Armstrong
Missouri University of Science and Technology

Karen D. Timmons

et. al. For a complete list of authors, see https://scholarsmine.mst.edu/chem_facwork/3597

Recommended Citation

J. I. Seeman et al., "Enantiomeric Resolution And Chiral Recognition Of Racemic Nicotine And Nicotine Analogues By B-cyclodextrin Complexation: Structure-enantiomeric Resolution Relationships In Host-guest Interactions," *Analytical Chemistry*, vol. 60, no. 19, pp. 2120 - 2127, American Chemical Society, Oct 1988.
The definitive version is available at https://doi.org/10.1021/ac00170a027

Enantiomeric Resolution and Chiral Recognition of Racemic Nicotine and Nicotine Analogues by β-Cyclodextrin Complexation. Structure–Enantiomeric Resolution Relationships in Host–Guest Interactions

Jeffrey I. Seeman* and Henry V. Secor

Philip Morris USA Research Center, P.O. Box 26583, Richmond, Virginia 23261

Daniel W. Armstrong,* Karen D. Timmons, and Timothy J. Ward

Department of Chemistry, University of Missouri—Rolla, Rolla, Missouri 65401

High-performance liquid chromatography using β-cyclodextrin bonded phases was examined for the enantiomeric separation of racemic nicotine and 19 racemic nicotine analogues. Ten pairs of enantiomers were separated by this technique. This represents the first reported facile and direct separation of these racemates. Effects of pH, mobile phase composition, and structural features of the substrates (i.e., position and size of substituents, presence of pyrrolidine ring or hydrogen bonding functionalities, basicity) on the enantioselectivity are examined. Various structural aspects of the compounds were related to retention to the cyclodextrin-modified support and to the enantiomeric separations observed. Implications of this work to host–guest complexation are discussed.

The chemistry of host–guest complexation has become a major factor in many areas of molecular recognition, including chromatography, catalysis, novel reaction media, and the study of weak interactions in biological systems (*1–3*). Tremendous advances have been made stemming from the original crown ethers of Pedersen (*4*) in the 1960s to the elegantly devised cryptands of Lehn (*5, 6*) and cavitands of Cram (*7–9*), among others, in the 1980s. In this work, we have investigated chiral recognition in host–guest systems using cyclodextrins (CD). Cyclic oligomers of the 1,4-glucopyranoside unit, cyclodextrins contain natural, chiral cavities. Because of their commercial availability, CDs are excellent candidates for studying chiral recognition in host–guest systems.

As part of a major effort into the use of high-performance liquid chromatography (HPLC) for both analytical and preparative enantiomeric separations (*10, 11*), we became interested in applying these techniques to tobacco alkaloids and related compounds. Our approach was to examine a set of compounds for which enantiomeric separation was observed, modify structural features of the substrates, and evaluate structure–resolvability relationships.

The synthesis and chemistry of nicotine and nicotine analogues are topics of much current interest (*12–15*). There has been reasonable success in the preparation of racemic nicotinoids (*12–22*). However, preparation of the optically pure isomers and analytical proof thereof remain considerable challenges (*23–25*). Reports continue to be published on the use of classical techniques (i.e., crystallization of diasteriomeric salts) for nicotine analogues (*26–28*) or the microbiological destruction of the naturally occurring enantiomer from a racemic mixture of nicotine (*29*). Recently, we have succeeded in preparing the optically pure enantiomers of nornicotine via preparative HPLC separation of their menthyloxycarbonyl (urethane) diasteriomers (*25*).

The separation and quantification of nicotine and a few related tobacco alkaloids and metabolites have been accomplished by thin-layer chromatography (TLC), gas chromatography (GC), and liquid chromatography (LC) (*30–42*). More extensive LC separations have been conducted on structural isomers and homologues of alkaloids related to nicotine (*43*). To our knowledge, other than our own current studies (*43, 44*), there have been no reports on the direct resolution of nicotine and/or nicotine analogue enantiomers by any chromatographic method. It is thus essential to develop methods that will both conveniently allow the experimental determination of optical purity and also provide a more general means of effecting their preparative resolution.

Herein, we examine the direct HPLC resolution of racemic nicotine (**1**) and 19 analogues by using β-cyclodextrin (β-CD) complexation (cf. Table I). This is the most extensive collection of racemic nicotinoids ever considered in a single work. As a result, much information can be obtained on the relationships between structure and chiral recognition. In particular, we have examined various steric, electronic, and conformational perturbations on enantiomeric resolution in this system.

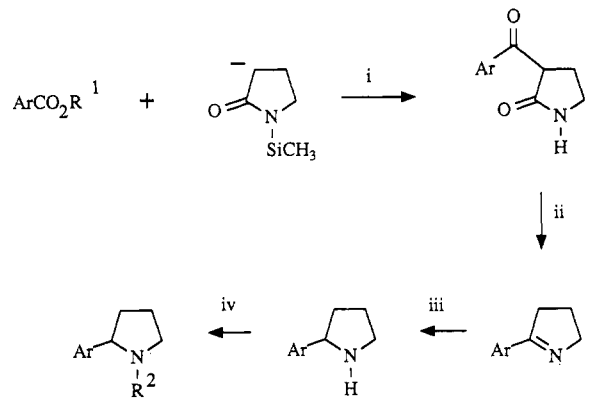
EXPERIMENTAL SECTION

Methods. All separations were done at room temperature (21 °C) with Shimadzu LC-4A and 6A liquid chromatographs. The compounds were detected at 254 nm with a variable-wavelength detector containing 13- and 8-μL flow cells, respectively. All samples were dissolved in acetonitrile or methanol (depending on the mobile phase composition) prior to injection. Typically, 5–10 μL of solution (0.1–0.5% nicotinoid) was injected. Columns (25 × 0.46 cm) containing β-cyclodextrin bonded to 5 μ silica were obtained from Advanced Separation Technologies, Whippany, NJ. The void volume of the column was determined by injecting neat methanol. The peak–trough combination caused by the change in refractive index was used as a marker. Flow rates, solvent compositions, and pH values are given in the respective tables and figures.

Materials. HPLC grade methanol, acetonitrile, triethylamine, and water were obtained from Fisher Scientific Co. Buffers were prepared by making a 1% solution of triethylamine in water and adding glacial acetic acid until the desired pH was obtained. *d,l*-Nicotine was prepared either by base-catalyzed racemization of (*S*)-(−)-nicotine (*45*) or by total synthesis (*46, 47*). Analogues **2**, **3**, **4**, and **20** were prepared by condensing *N*-(trimethylsilyl)-2-pyrrolidinone with the appropriate carboxylic acid ester followed by acid-catalyzed hydrolysis, decarboxylation, reduction, and methylation as described previously (*46–48*). The fluoronicotinoids **7** and **8** and the alkylnicotinoids **18** and **19** were reported previously by us (*47, 49, 50*). *N'*-Ethylnornicotine (**6**) was prepared by sodium borohydride reduction of *N'*-acetyl-

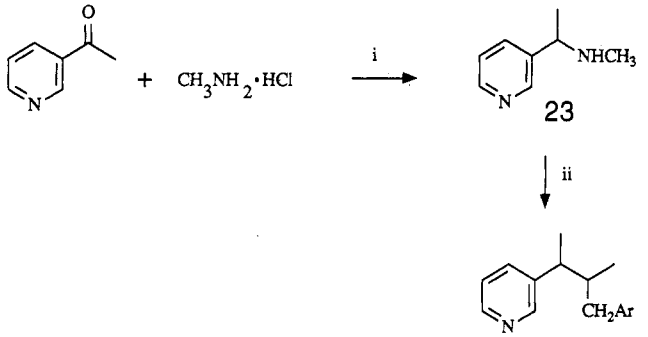
0003-2700/88/0360-2120$01.50/0

Scheme I[a]



[a] i, Lithium diisopropylamide; ii, hydrochloric acid, heat, then hydroxide; iii, sodium cyanoborohydride; iv, *n*-butyllithium, then R^2Br.

Scheme II[a]



[a] i, Sodium cyanoborohydride; ii, *n*-butyllithium, then $ArCH_2Br$.

nornicotine, the latter prepared by treatment of nornicotine with acetyl chloride (*51*). Analogues **9–13** were each made according to the chemistry shown in Scheme I and **14–16** as shown in Scheme II (*46*, *47*, *52–54*). Full details for the preparation of the new compounds are available as supplementary material.

RESULTS AND DISCUSSION

A variety of racemates have been resolved by LC using cyclodextrin bonded phases developed in our laboratory (*10*, *11*, *55–59*). Cyclodextrin-containing mobile phases also have been used in conjunction with achiral stationary phases to resolve some isomeric mixtures (*60–62*). Interestingly, there seem to be a few compounds for which resolution is easily achieved by one technique but not the other, even though the same cyclodextrin is used.

Table I gives the structure of nicotine and 19 chiral analogues examined in this study. The effect of structure on chiral recognition was examined by using these systematically altered nicotine analogues. Note that the structural changes fall into three general classes. The simplest modification examined was the nature of the aromatic ring (cf. **1–4**). In another group of compounds (**6–13**, **17**), the pyrrolidine nitrogen substituent was altered. Variations in size, electron-withdrawing ability, hydrogen-bonding ability, and/or position of covalent bonding were incorporated. A third group of compounds was analogous to those in the second group except that the pyrrolidine ring was opened (**5**, **14–16**). In the last group, substituents were added to the pyridine ring (**18–20**).

Table I also lists the separation data for the racemic nictinoids that were resolved by using a β-cyclodextrin bonded phase column. Note that two different mobile phase regimes were employed. The best separations were obtained with hydro–organic mobile phases with acetonitrile as the organic modifier. The aqueous portion of the mobile phase was buffered at a pH of 7.1 with triethylammonium acetate. When

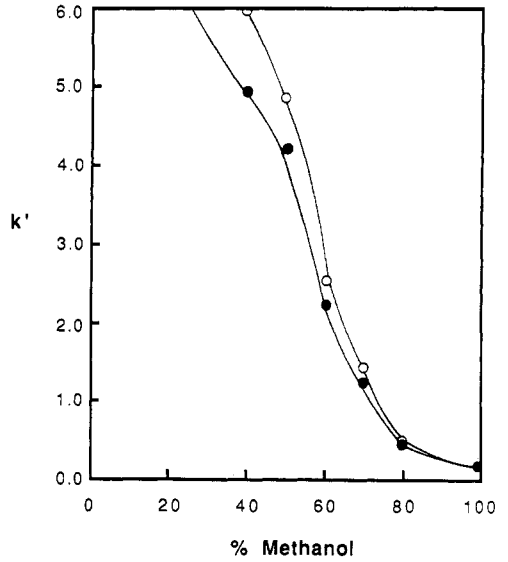


Figure 1. Plot of capacity factors versus methanol content of the mobile phase for racemic *N'*-benzylnornicotine. Note the difference in shape and location of this curve versus the analogous plot for acetonitrile (see Figure 2). The open circles (O) are for the (*R*)-(+)-enantiomer and the closed circles (●) are for the (*S*)-(−)-enantiomer.

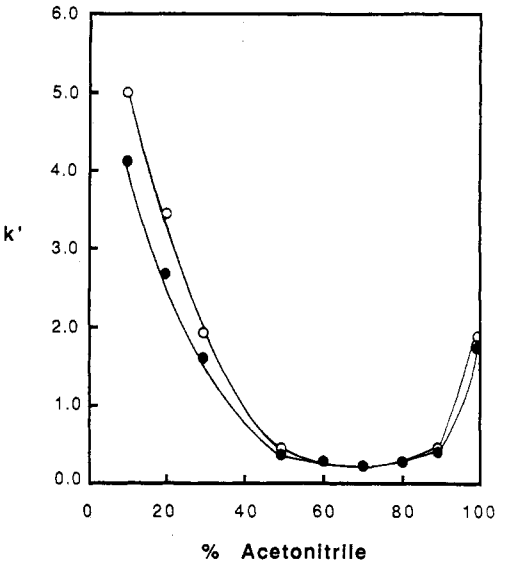


Figure 2. Plots showing the effect of mobile phase composition (i.e., % acetonitrile) on the enantiomeric resolution of racemic *N'*-benzylnornicotine. Note that there are two different types of behavior at high modifier concentration. (See Figure 1 for the first type.) The minima in this curve may indicate a change in the mechanism of chiral recognition. The open circles (O) are for the (*R*)-(+)-enantiomer and the closed circles (●) are for the (*S*)-(−)-enantiomer.

the pH of the mobile phase was decreased, enantiomeric resolution decreased. At pH <5.0, no enantiomeric separation was observed for any of the nicotinoids in this study.

An unusual and remarkable type of retention behavior was observed for approximately half of the nicotinoids when acetonitrile was used as the organic modifier. A retention minimum was reached between 60% and 80% (by volume) acetonitrile. Further increases in acetonitrile concentration (up to 100% organic modifier) caused increased retention. Similar minima have been observed on achiral reversed phase columns and attributed to either residual silanol groups or solubility limitations of the solute. However, in this study *enantiomeric resolution also increased* at high acetonitrile concentration (see *N'*-benzylnornicotine in Figures 1 and 2). As the bonded β-cyclodextrin is the only chiral constituent of the column, it must be responsible for the observed be-

Table I. Resolution, Selectivity, and Retention Data for Racemic Nicotine and Racemic Nicotine Analogues (2–20)

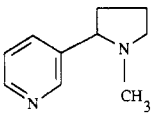
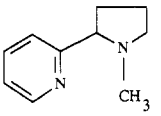
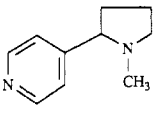
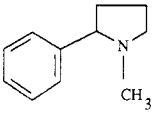
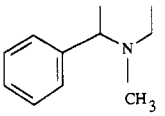
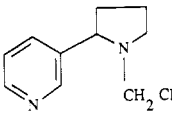
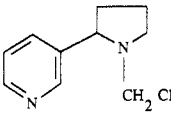
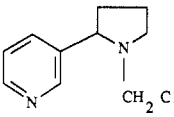
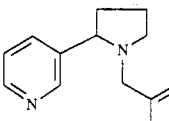
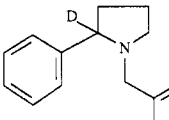
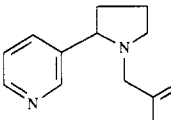
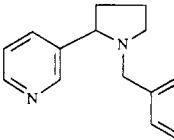
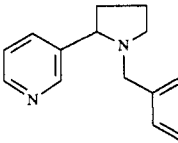
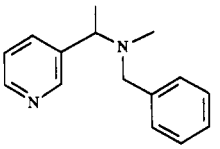
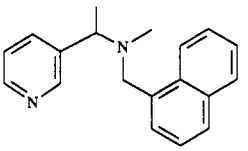
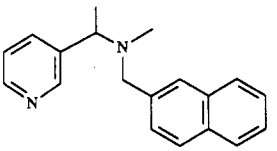
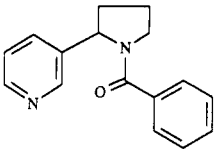
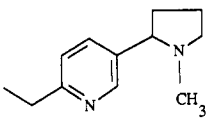
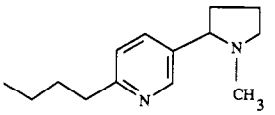
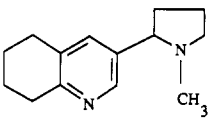
no.	compound		k'^a	α^b	R_S^c	mobile phase[d]	flow rate[e]	column[f]
1	nicotine					g		
2	1-methyl-2-(2-pyridyl)pyrrolidine					g		
3	1-methyl-2-(4-pyridyl)pyrrolidine					g		
4	1-methyl-2-phenylpyrrolidine					g		
5	*N*-ethyl-*N*,α-dimethylphenylmethanamine					g		
6	*N'*-ethylnornicotine					g		
7	*N'*-(2,2-difluoroethyl)nornicotine		3.86 0.60	1.03 1.00	1.14	10/90 100.0	1.0 1.0	II II
8	*N'*-(2,2,2-trifluoroethyl)nornicotine		1.03 0.64	1.09 1.19	1.51 1.29	25/75 100/0	1.0 1.0	II II
9	*N'*-benzylnornicotine		2.82 2.23	1.18 1.14	2.36 1.47	30/70 100/0	1.0 1.0	I I
10	1-benzyl-2-phenylpyrrolidine[h]		6.70 4.48	1.02 1.00	0.30	10/90 100/0	0.5 1.0	I I
11	*N'*-(2-methylbenzyl)nornicotine		8.13 1.22	1.07 1.15	1.54 1.17	10/90 100/0	0.8 1.0	I I
12	*N'*-(1-naphthylmethyl)nornicotine					g		
13	*N'*-(2-naphthylmethyl)nornicotine		5.35 3.37	1.07 1.02	2.02 1.12	30/70 100/0	0.8 0.8	I I

Table I (Continued)

no.	compound		k'^a	α^b	R_S^c	mobile phase[d]	flow rate[e]	column[f]
14	*N*-benzyl-*N*,α-dimethyl-3-pyridinemethanamine		2.05	1.03	0.80	20/80	0.5	I
			2.53	1.00		100/0	0.5	I
15	*N*,α-dimethyl-*N*-(1-naphthylmethyl)-3-pyridinemethanamine					*g*		
16	*N*,α-dimethyl-*N*-(2-naphthylmethyl)-3-pyridinemethanamine		1.64	1.09	1.21	20/80	0.5	I
			2.83	1.00		100/0	0.8	I
17	*N'*-benzoylnornicotine		4.75	1.05	1.32	5/95	0.5	I
			4.06	1.00		100/0	1.0	I
18	6-ethylnicotine					*g*		
19	6-butylnicotine					*g*		
20	5,6-cyclohexenonicotine		2.97	1.04	1.50	25/75	1.0	II
			4.16	1.07	1.57	100/0	1.0	II

[a] The capacity factor corresponds to the first eluting enantiomer. [b] α is the separation factor and is equal to the ratio of one enantiomer's k' to the other enantiomer's k'. [c] R_s is the resolution and is equal to 2 $(t_2 - t_1)/(w_1 + w_2)$ where t and w are the peak retention times and peak widths, respectively. [d] The mobile phase consisted of acetonitrile/aqueous triethylammonium acetate (pH 7.1). [e] mL/min. [f] I, one 25-cm β-cyclodextrin column; II, two 25-cm β-cyclodextrin columns connected in series. [g] Not resolved. [h] Nicotinoid **10** used in this study was synthesized in such a manner to incorporate a deuterium atom at C-2. The deuterium should have no effect on the enantiomeric resolution obtained in this work.

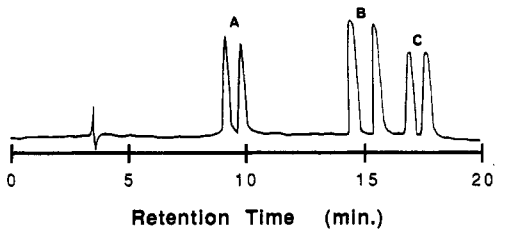
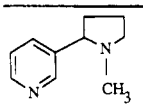
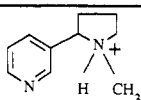
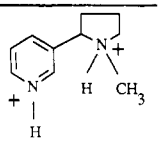


Figure 3. Chromatogram showing the separation of the following racemic nicotinoids on a 25-cm β-CD column: (A) *N'*-(2,2,2-trifluoroethyl) nornicotine (**8**) (R_S = 1.5), (B) *N'*-benzylnornicotine (**9**) (R_S = 1.9), and (C) *N'*-(2-naphthylmethyl)nornicotine (**16**) (R_S = 1.5). This particular separation used gradient elution from 10:90 (v:v) acetonitrile:1% aqueous triethylammonium acetate (pH 7.1) to 70:30 in 20 min. The flow rate was 1.0 mL/min and the wavelength of detection was 254 nm.

havior. This change in retention behavior (Figure 2) may indicate a change in the mechanism of chiral recognition. Currently, it is not known if retention is due solely to interactions between the external hydroxyls and the nicotinoid (as in a normal-phase separation) or if some kind of complexation is involved. Interestingly, the elution order of the enantiomers of *N'*-benzylnornicotine (**9**) is identical in hydro–organic

Table II. Relative Concentrations of Nicotine Free Base and Mono- and Diprotonated Species as a Function of pH

pH	relative concentration,[a] %		
			
7.1	10.7	89.3	
5.0	0.1	98.7	1.2

[a] Calculated from $pK_a(N') = 8.02$, $pK_a(N) = 2.85$.

mobile phases and 100% acetonitrile, as the (*R*)-(+)-enantiomer elutes before the (*S*)-(−)-enantiomer in both cases. A typical separation of three racemic nicotinoids is shown in Figure 3.

It is evident from the data in Table I that several different factors are involved in achieving chiral recognition in β-cyclodextrin systems. Among these controlling features are steric and electronic effects and hydrogen-bonding capabilities.

As shown in Table II, the predominant species present in an aqueous solution of nicotine at pH 5.0–7.1 is the mono-

Table III. Comparison of Basicity of Amines

compound	pK_{a1}	pK_{a2}	ref
nicotine (1)	8.0	2.9	*a*
1-methyl-2-phenylpyrrolidine (4)	9.3		*a*
N'-(2,2-difluoroethyl)nornicotine (7)	5.1	2.3	*b*
ethylamine	10.8		*c*
2-fluoroethylamine	8.8		*d*
2,2-difluoroethylamine	7.1		*d*
2,2,2-trifluoroethylamine	5.6		*d*

[a] Reference 65. [b] Reference 49. [c] Reference 66. [d] Reference 67.

protonated salt. However, at pH 7.1, a substantial concentration of free base is also present. As the rates of interconversion from one form to the other are extremely fast at these acidities, a Curtin–Hammett analysis (*63*, *64*) clearly indicates that all forms could be involved in the chiral recognition phenomena. However, since enantiomeric separations were not observed at pH <5.0, we conclude (cf. Table II) that the nicotinoid free base is the species predominantly involved in the chiral recognition step.

Neither of the unsubstituted 1-methyl-2-arylpyrrolidines **1–4** nor the ring cleaved **5** shows any substantial resolution under a wide range of conditions examined in this work, although nicotine itself demonstrated some line broadening with a hint of a shoulder under optimum conditions.

Racemic *N'*-benzylnornicotine (**9**) was resolved to the largest extent among the compounds examined herein. This is likely to be due to two phenomena: the presence of the phenyl ring that results in a tighter inclusion complex, and complexation to the nitrogen atom of its pyridine ring. Support for these conclusions comes from the lack of resolution of **1** and **6** and from the poor resolution observed for the structurally very similar 1-benzyl-2-phenylpyrrolidine (**10**). The relative unimportance of complexation to the pyrrolidine ring nitrogen (N') atom is further displayed by the resolution observed for the amide *N'*-benzoylnornicotine (**17**), the N' of **17** being far less basic than that of **9** but near base-line resolution still obtained. Note, however, that the pyrrolidine nitrogen of **9** is more basic than that of **17**, and **9** is significantly better resolved than **17**.

While *N'*-ethylnornicotine (**6**) was not resolved, its fluorinated analogues **7** and **8** were separated into their enantiomers. The greater the number of fluorine atoms, the better the chiral recognition and resolution. In fact, the trifluoro analogue **8** was base-line resolved. Fluorine has essentially the same steric size as a hydrogen atom, so size alone is not controlling. We previously have shown that **6–8** have essentially identical conformations in solution (by careful ^1H NMR studies) and in gas phase (by ab initio calculations) (*49*). As shown in Table III, the presence of the fluorine atoms significantly alters the basicity (pK_a) of the pyrrolidine nitrogen. These results further support our conclusion (cf. above) that the pyrrolidine nitrogen is unprotonated in the chiral recognition state, since the fluorine atoms act to decrease the basicity of the pyrrolidine nitrogen atom. However, in these cases, the less basic nicotinoids are resolved better. We thus suggest that the fluorine atoms act as hydrogen bonding acceptors to enhance chiral recognition (*68*).

The crucial importance of steric effects and minor structural variations is best illustrated by comparing the resolutions of **9** and **11–13**. The 2-naphthyl derivative **13** has exceptionally good resolution (R_S = 2.02) while the closely related 1-naphthyl nicotinoid **12** shows absolutely no chiral recognition even though it is well retained by the column. That substitution at the ortho position of the phenyl ring in **9** hinders chiral recognition (cf. **12** vs **13**) is further highlighted by the lower resolution of **11** compared to **9**. Analogous phenomena were observed previously for 1- and 2-naphthyl and naphthylamide derivatized amino acids (*55*, *56*).

Interestingly, the presence of the pyrrolidine ring in these analogues is not an absolutely essential structural feature for chiral recognition by the β-CD. The "ring cleaved" analogues **14** (to **9**) and **16** (to **13**) are all resolved, while the 1-naphthyl analogue **15** (to **12**) is not.

Finally, addition of alkyl groups to the pyridine ring of nicotine (**18** and **19**) did not enhance the separation of unresolved nicotine. However, the cyclic analogue of 6-butylnicotine, namely 5,6-cyclohexenonicotine (**20**), was base-line resolved. It is tempting to speculate that the base-line resolution of **20** relative to the lack of resolution of **19** is due to the cyclic, more spherical nature of the former molecule. Chiral recognition may not be enhanced by the presence of long hydrocarbon chains that have sufficient mobility (i.e., low-energy conformations available to it) to assume many conformations within a β-CD cavity.

For enantiomeric resolution to occur, one enantiomer of a racemate must form a more stable complex with the "host" substrate than its antipode. Evaluation of the data in Tables I–III and consideration of the arguments proposed above suggest that at least two important, different complexation mechanisms are operating. In one, complexation is enhanced by a nonbonded interaction with the π system of N'-substituted phenyl ring of **9–11**, **13** and **14**, and **16** and **17**. Steric effects are present, however, that can render this interaction unfavorable, as noted in the case of **11** and probably **12** and **15**. In the second, complexation is enhanced by a hydrogen-bonding interaction between the β-CD and the fluorine atoms of **7** and **8** (compare these substrates with **1–6**).

Three parameters characterize these HPLC separations. The capacity factor k' reflects the retention of the substrate; the separation factor α reflects the relative retentions of the enantiomers and is equal to the ratio of one enantiomer's k' to the other enantiomer's k', and the resolution R_s takes into consideration the retention properties and peak widths of both enantiomers. It is interesting to speculate why some racemates can be resolved by using these techniques while other, very similar racemates cannot. Thus, the 1-naphthylnornicotine **12** does not resolve while the 2-naphthylnornicotine **13** does resolve.

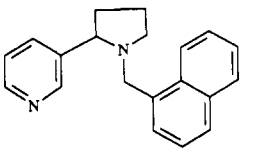
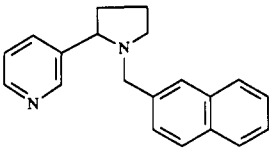
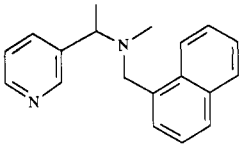
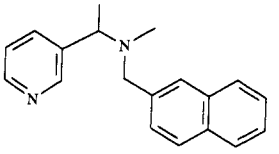
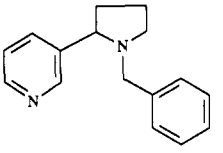
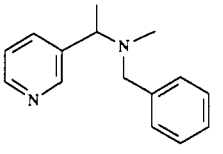
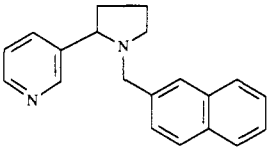
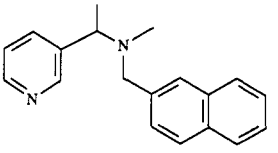
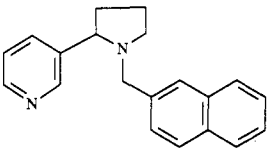
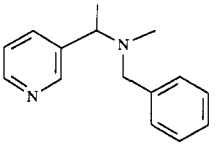
Note that there often seems to be an empirical relationship between retention (k') and enantioselectivity (α) for structurally related racemates. That is, the racemate exhibiting the greater resolution often has the greater retention. Table IV gives several examples in which each comparison represents analyses performed on the same column under identical operating conditions, one after the other.

Consider the first five comparisons shown in Table IV. In each case, a pair of structurally related racemates is examined (comparisons 1 and 2, 1- vs 2-naphthyl; comparisons 3–5, pyrrolidine ring vs acyclic molecule). In these five comparisons, the better the separation (i.e., the larger of the two α values), the greater the values of the k'.

To illustrate the subtleties of this logic, the sixth comparison in Table IV does not follow the trend found for this first five comparisons. In comparison 6, the better enantiomeric resolution is found for *d,l*-**13** (1.09 vs 1.03) but **14** is retained more strongly (2.05 and 2.11 vs 1.64 and 1.79). Importantly, **13** and **14** are structurally dissimilar in two ways: the nature of the N' substituent (phenyl vs 2-naphthyl) and pyrrolidine ring vs acyclic structure. We thus make the very reasonable conclusion that the relationship between α and k' must be for structurally similar substrates that are complexed in an analogous manner, else additional binding mechanisms will occur which cloud the resolution–retention relationship.

Caution must be exercised in making comparisons such as these. It is known that there are a variety of interactions

Table IV. Comparison of Retention of Enantiomers with Enantiomeric Separation[a]

comparison	compound		k'	α
1[b]	12		1.3	
	13		2.0, 2.16	1.14
2[c]	15		3.70	
	16		8.36, 8.58	1.03
3[d]	9		2.67, 2.95	1.10
	14		2.17, 2.20	1.01
4[e]	9		3.21, 3.63	1.13
	14		2.60, 2.65	1.02
5[d]	13		5.35, 5.71	1.07
	16		3.01, 3.08	1.02
6[c]	13		1.64, 1.79	1.09
	14		2.05, 2.11	1.03

[a] See Table I for definition of k' and α. The mobile phase consisted of acetonitrile/aqueous triethylammonium acetate (pH 7.1). [b] 40% acetonitrile/60% buffer. [c] 20% acetonitrile/80% buffer. [d] 30% acetonitrile/70% buffer. [e] 25% acetonitrile/75% buffer.

between a racemic substrate and a CSP and that only a few of these are enantioselective (*69*). In order for the k':α relationship to be valid, all of the major interaction parameters leading to retention must be similar except for one or more of the enantioselective interactions. It is entirely possible that two slightly dissimilar racemates could show the opposite k':α relationship if their respective inclusion complexes with β-CD were very different.

We have recently found that a few of these enantiomers (**1**–**4**, **7**–**9**, **13**, and **20**) can be separated on achiral LC microcolumns with cyclodextrin-containing mobile phases (*44*). In this case, the cyclodextrin is both adsorbed on the stationary phase and present as a mobile phase carrier molecule. The bonded chiral phase approach has been generally thought to be superior for solutes that could be resolved by both methods. This was because the "chiral mobile phase additive approach" tended to produce broad peaks (poor efficiency) and required longer columns and separation times. The most significant thing about the β-CD mobile phase technique was that a few of the racemates (including nicotine) could be resolved by this approach *despite* the lack of success on the β-CD bonded phase. Compounds **1**–**4** in particular gave good resolution with β-CD mobile phases while showing little or no chiral recognition with β-CD bonded phases. The reasons for this are not entirely clear but may be related to changes in the binding ability of the bonded phase and to the multiple equilibria possible when β-CD is in free solution (*3*).

CONCLUSIONS

β-Cyclodextrin shows a high degree of chiral recognition for a variety of optically active nicotine analogues. The enantiomer separations can be extremely sensitive to minor structural variations. The enantiomeric separations of the various racemates appear to be related to the capacity factors k' of the individual enantiomers, i.e., to the strength of the complexation. At least two specific types of β-CD interactions can be postulated: with the π system of pendant aromatic rings and with the fluorine atoms of alkyl substituents. Because of the range of structural modifications employed, steric and hydrogen bonding factors in these host–guest systems can be postulated. This represents the first resolutions of racemic nicotine analogues using routine and facile analytical methodologies. Some nicotinoids such as *N'*-benzylnornicotine show retention and resolution minima as a function of organic modifier concentration on β-cyclodextrin bonded phases. These minima may indicate a change in the mechanism of chiral recognition at different mobile phase compositions.

ACKNOWLEDGMENT

We thank W. F. Kuhn, R. N. Ferguson, and Y. Houminer for their encouragement and support of this work, R. L. Bassfield and J. Campbell for obtaining NMR data, C. G. Chavdarian for preparing nicotinoid **20**, B. Good for computer software development, and T. Sinkiewicz for secretarial assistance.

Registry No. **1**, 22083-74-5; **2**, 79733-05-4; **3**, 71606-36-5; **4**, 2512-64-3; **5**, 115459-13-7; **6**, 86900-39-2; **7**, 104165-35-7; (*R*)-**7**, 115459-17-1; (*S*)-**7**, 112791-59-0; **8**, 104165-36-8; (*R*)-**8**, 115459-18-2; (*S*)-**8**, 112791-60-3; **9**, 75652-51-6; **9** dipicrate, 115384-02-6; (*R*)-**9**, 115459-19-3; (*S*)-**9**, 2055-30-3; **10**, 115383-94-3; **10** picrate, 115384-03-7; (*R*)-**10**, 115459-20-6; (*S*)-**10**, 115459-21-7; **11**, 115383-95-4; (*R*)-**11**, 115459-22-8; (*S*)-**11**, 115459-23-9; **12**, 115383-96-5; **13**, 115383-97-6; (*R*)-**13**, 115459-24-0; (*S*)-**13**, 115459-25-1; **14**, 115383-98-7; (*R*)-**14**, 115459-26-2; (*S*)-**14**, 115459-27-3; **15**, 115383-99-8; **16**, 115384-00-4; (*R*)-**16**, 115459-28-4; (*S*)-**16**, 115459-29-5; **17**, 115460-89-4; (*R*)-**17**, 115406-80-9; (*S*)-**17**, 5979-95-3; **18**, 115459-14-8; **19**, 115459-15-9; **20**, 115459-16-0; **20** dipicrate, 115459-32-0; (*R*)-**20**, 115384-01-5; (*S*)-**20**, 112727-17-0; **23**, 115459-30-8; **23** dipicrate, 115459-31-9; **24**, 94815-18-6; **25**, 115384-04-8; **26**, 115384-05-9; **26** dipicrate, 115384-06-0; (*R,S*)-nornicotine, 5746-86-1; benzyl bromide, 100-39-0; (*R,S*)-*N*,α-dimethylbenzylamine, 42882-26-8; bromoethane, 74-96-4; 2-phenylpyrrolidine, 1006-64-0; 2-bromomethyltoluene, 89-92-9; 1-bromomethylnaphthalene, 3163-27-7; 2-bromomethylnaphthalene, 939-26-4; 3-acetylpyridine hydrochloride, 67210-10-0; methylamine hydrochloride, 593-51-1; ethyl(ethoxymethylene)cyanoacetate, 94-05-3; 1-pyrrolidino-1-cyclohexene, 1125-99-1; *N*-trimethylsilyl-2-pyrrolidinone, 14468-90-7; sodium cyanoborohydride, 25895-60-7; formaldehyde, 50-00-0.

Supplementary Material Available: Details for the preparation of compounds **5**, **9**–**16**, **20**, and **23**–**26** (11 pages). Photocopies of the supplementary material from this paper or microfiche (105 × 148 mm, 24× reduction, negatives) may be obtained from Microforms & Back Issues Office, American Chemical Society, 1155 16th Street, NW, Washington, DC 20036. Orders must state whether for photocopy or microfiche and give complete title of article, names of authors, journal issue date, and page numbers. Prepayment, check or money order for $22.00 for photocopy ($24.00 foreign) or $10.00 for microfiche ($11.00 foreign), is required and prices are subject to change.

LITERATURE CITED

(1) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin, 1978.
(2) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademai Klado: Budapest, 1982.
(3) Armstrong, D. W.; Nome, F.; Spino, L. A.; Golden, T. D. *J. Am. Chem. Soc.* **1986**, *108*, 1418.
(4) Pedersen, C. J. *J. Am. Chem. Soc.* **1967**, *89*, 2495.
(5) Lehn, J.-M. *Science (Washington, D.C.)* **1985**, *227*, 849.
(6) Lehn, J.-M. *N.Y. Acad. Sci.* **1986**, *471*, 41.
(7) Cram, D. J. *Science (Washington, D.C.)* **1983**, *219*, 1177.
(8) Cram, D. J.; Cram, J. M. "Designed Complexes - Science and Applications," In *Selectivity; A Goal for Synthetic Efficiency*; Bartmann, W., Trost, B. M., Eds.; Workshop Conference Hoechst; Verlag Chemie: Weinheim, Germany, 1983; Vol. 14, pp 42–64.
(9) Cram, D. J. "From Discovery to Design in Organic Chemistry"; *Profiles, Pathways and Dreams. The Development of Modern Organic Chemistry*; Seeman, J. I., Ed.; American Chemical Society: Washington, DC, in press.
(10) Armstrong, D. W. *Anal. Chem.* **1987**, *59*, 84A.
(11) Armstrong, D. W.; Li, W. *Chromatogr. Forum* **1987**, *2*, 43.
(12) Seeman, J. I. *Heterocycles* **1984**, *22*, 165.
(13) Strunz, G. M.; Findlay, J. A. *The Alkaloids*; Brossi, A., Ed.; Academic: New York, 1985; Vol. 26, Chapter 3, p 89.
(14) Seeman, J. I. *Pure Appl. Chem.* **1987**, *59*, 1661.
(15) Kisaki, T.; Maeda, S.; Koiwai, A.; Mikami, Y.; Sasaki, T.; Matsushita, H. *Beitr. Tabakforsch. Int.* **1976**, *9*, 308.
(16) Rondahl, L. *Acta Pharm. Suec.* **1980**, *17*, 347.
(17) Langhals, E.; Langhals, H.; Rüchardt, C. *Justus Liebigs' Ann. Chem.* **1983**, 330.
(18) Shibagaki, M.; Matsushita, H.; Kaneko, H. *Heterocycles* **1985**, *23*, 1681.
(19) Acheson, R. M.; Ferris, M. J.; Sinclair, N. M. *J. Chem. Soc., Perkin Trans. 1* **1980**, 78.
(20) Chavdarian, C. G.; Seeman, J. I.; Wooten, J. B. *J. Org. Chem.* **1983**, *48*, 492.
(21) Seeman, J. I.; Chavdarian, C. G.; Secor, H. V.; Osdene, T. S. *J. Org. Chem.* **1986**, *51*, 1548.
(22) Catka, T. E.; Leete, E. *J. Org. Chem.* **1978**, *43*, 2125.
(23) Chavdarian, C. G. *J. Org. Chem.* **1983**, *48*, 1529.
(24) Chavdarian, C. G.; Sanders, E. B.; Bassfield, R. L. *J. Org. Chem.* **1982**, *47*, 1069.
(25) Seeman, J. I.; Chavdarian, C. G.; Secor, H. V. *J. Org. Chem.* **1985**, *50*, 5419.
(26) Kachur, J. F.; May, E. L.; Awaya, H.; Egle, J. L., Jr.; Aceto, M. D.; Martin, B. R. *Life Sci.* **1986**, *38*, 323.
(27) Jacob, P., III *J. Org. Chem.* **1982**, *47*, 4165.
(28) Vincek, W. C.; Martin, B. R.; Aceto, M. D.; Tripathi, H.; May, E. L.; Harris, L. S. *J. Pharm. Sci.* **1981**, *70*, 1292.
(29) Edwards, W. B., III; McCuen, R. *J. Org. Chem.* **1983**, *48*, 2484, and references cited therein.
(30) Barz, W.; Kettner, M.; Husemann, W. *Planta Med., J. Med. Plant Res.* **1978**, *34*, 73.
(31) Beckett, A. H.; Triggs, E. J. *Nature (London)* **1966**, *211*, 1415.
(32) Berry, D. J.; Grove, J. *J. Chromatogr.* **1971**, *61*, 111.
(33) Bluthgen, V. A.; Nijhuis, H.; Hamann, J.; Heeschen, W. *Milchwissenschaft* **1978**, *33*, 137.
(34) Burns, D. T.; Collin, E. J. *J. Chromatogr.* **1977**, *133*, 378.
(35) Bush, L. P. *J. Chromatogr.* **1972**, *73*, 243.
(36) Clarke, E. G. C.; Hawkins, A. E. *J. Forensic Sci. Soc.* **1961**, *1*, 120.
(37) Watson, I. D. *J. Chromatogr.* **1977**, *143*, 203.
(38) Plade, J. J.; Hoffmann, D. *J. Liq. Chromatogr.* **1980**, *3*, 1505.
(39) Saunders, J. A.; Blume, D. E. *J. Chromatogr.* **1981**, *205*, 147.
(40) Sudan, B. J. L.; Brouillard, C.; Strehler, C.; Strub, H.; Sterboul, J.; Sainte-Laudy, J. *J. Chromatogr.* **1984**, *288*, 415.
(41) Cundy, K. C.; Crooks, P. A. *J. Chromatogr.* **1984**, *306*, 291.
(42) Mousa, S.; Van Loon, G. R.; Houdi, A. A.; Crooks, P. A. *J. Chromatogr.* **1985**, *347*, 405.

(43) Armstrong, D. W.; Seeman, J. I. et al., unpublished results, 1987.
(44) Armstrong, D. W.; Spino, L. A.; Han, S. M.; Seeman, J. I.; Secor, H. V. J. Chromatogr. 1987, 411, 490.
(45) Tsujino, Y.; Shibata, S.; Katsuyama, A.; Kisaki, T.; Kaneko, H. Heterocycles 1982, 19, 2151.
(46) Hu, M. W.; Bondinell, W. E.; Hoffmann, D. J. Labelled Compd. 1974, 10, 79.
(47) Seeman, J. I.; Secor, H. V.; Chavdarian, C. G.; Sanders, E. B.; Bassfield, R. L.; Whidby, J. F. J. Org. Chem. 1981, 46, 3040.
(48) Glenn, D. F.; Edwards, W. B., III, J. Org. Chem. 1978, 43, 2860.
(49) Cox, R. H.; Kao, J.; Secor, H. V.; Seeman, J. I. J. Mol. Struct. 1986, 140, 93.
(50) Seeman, J. I.; Chavdarian, C. G.; Kornfeld, R. A.; Naworal, J. D. Tetrahedron 1985, 41, 595.
(51) Mattila, M.; Vartiainen, A. Ann. Med. Exp. Biol. Fenn. 1964, 42, 27.
(52) Abood, L. G.; Reynolds, D. T.; Bidlack, J. M. Life Sci. 1980, 27, 1307.
(53) Seeman, J. I. Synthesis 1977, 498.
(54) Borch, R. F.; Bernstein, M. D.; Durst, H. D. J. Am. Chem. Soc. 1971, 93, 2897.
(55) Armstrong, D. W.; DeMond, W. J. Chromatogr. Sci. 1984, 22, 411.
(56) Armstrong, D. W.; Alak, A.; Bui, K. H.; DeMond, W.; Ward, T.; Riehl, T. E.; Hinze, W. L. J. Inclusion Phenom. 1984, 2, 533.
(57) Armstrong, D. W.; DeMond, W.; Czech, B. P. Anal. Chem. 1985, 57, 481.
(58) Armstrong, D. W.; Ward, T. J.; Czech, A.; Czech, B. P.; Bartsch, R. A. J. Org. Chem. 1985, 50, 5556.
(59) Armstrong, D. W.; Ward, T. J.; Armstrong, R. D.; Beesley, T. E. Science (Washington, D.C.) 1986, 232, 1132.
(60) Hinze, W. L.; Armstrong, D. W. Anal. Lett. 1980, 13, 1093.
(61) Degowski, J.; Sybilska, D.; Juraczak, J. J. Chromatogr. 1982, 237, 303.
(62) Sybilska, D.; Zukowskki, J.; Bojarski, J. J. Liq. Chromatogr. 1986, 9, 591.
(63) Seeman, J. I. Chem. Rev. 1983, 83, 83.
(64) Seeman, J. I. J. Chem. Educ. 1986, 63, 42.
(65) Seeman, J. I.; Whidby, J. F. J. Org. Chem. 1976, 41, 3824.
(66) Love, R.; Cohen, R. B.; Taft, R. W. J. Am. Chem. Soc. 1968, 90, 2455.
(67) Perrin, D. D. Dissociation Constants of Organic Bases in Aqueous Solution; Butterworths: London, 1965.
(68) Vinogradov, S. N.; Linnell, R. H. Hydrogen Bonding; Van Nostrand Reinhold: New York, 1971; p 14.
(69) Boehm, R. E.; Martire, D. E.; Armstrong, D. W. Anal. Chem. 1988, 60, 522.

RECEIVED for review August 17, 1987. Resubmitted December 29, 1987. Accepted April 20, 1988. Support of a portion (to D.W.A.) of this work by the Smokeless Tobacco Research Council, Inc. (Grant 0154), is gratefully acknowledged.

Cyclodextrin-Modified Solvent Extraction for Polynuclear Aromatic Hydrocarbons

Lisa A. Blyshak, Thomas M. Rossi,[1] Gabor Patonay,[2] and Isiah M. Warner*

Department of Chemistry, Emory University, Atlanta, Georgia 30322

The extraction efficiencies of several polynuclear aromatic hydrocarbons (PAHs) between isopropyl ether/water and between isopropyl ether:1-butanol (1:4)/water are measured in the presence of an aqueous γ-cyclodextrin (CDx) modifier at room temperature. The distribution of certain PAHs into the aqueous phase is increased by the presence of 10^{-2} M γ-CDx. For compounds such as perylene and coronene, which show the most marked effects, the extraction efficiencies into the aqueous phase from pure isopropyl ether are 95.1% and 93.7%, respectively, when the CDx modifier is used. In the mixed solvent system with 1-butanol, these values are 63.4% and 98.1%, respectively. In both systems, the increased distribution into water is based in part on the size relationship between the PAH and the CDx cavity. In the case of relatively small molecules like anthracene, little or no extraction is observed in the presence of the CDx modifier. This type of extraction system may be useful for selective extraction of large PAHs from mixtures. Extraction results for a variety of PAHs are presented and discussed.

In recent years, the analytical utility of cyclodextrins has become increasingly evident. These compounds, which are cyclic oligosaccharides capable of forming inclusion complexes (*1*, *2*), have been evaluated for use in several analytical systems. The most commonly used oligosaccharides are α-, β-, and γ-cyclodextrins that have approximate inner cavity diameters of 5.0, 7.8, and 9.5 Å, respectively. The degree of complex formation between host and guest is closely related to the compatibility of the CDx cavity size with the size and steric arrangement of the potential guest and to the hydrophobicity of the potential guest (*1*). Since complex formation involves a stereoselective interaction that affords some measure of protection for the included species, these macrocycles have become useful in fluorescence and phosphorescence enhancement (*3–5*), stereoselective catalysis (*6*), and reversed-phase chromatographic separations (*7*, *8*).

In general, appreciable extraction of PAHs into the aqueous phase is not feasible because the solubilities of most PAHs in water are very low. In cases where extraction from a nonpolar organic phase to polar phase is desired, a polar organic solvent such as dimethyl sulfoxide (DMSO) is used to extract polycyclic aromatics from hydrocarbon solvents (*9*). Solvent extraction schemes requiring polar solvents such as these are simple and are useful for removing PAHs from organic matrices but are limited in their selective interactions with particular PAHs. The solvent extraction scheme described in this paper shows that the use of CDx as an aqueous phase modifier enhances the extraction of selected species into the aqueous layer while retaining other species in the bulk organic phase. The extraction efficiency is related to the cyclodextrin complexation and thus to the size and hydrophobicity of the compounds to be extracted.

This method may be particularly useful for simplifying complex mixtures of organic material such as oil samples or air sample adsorbates, which are usually soluble in organic solvents. Such samples contain a variety of PAHs, which would make them amenable to separations by extraction into aqueous phase. The use of cyclodextrins would allow sim-

[1] Present address: Smith, Kline & French Laboratories, Philadelphia, PA 19101.
[2] Present address: Department of Chemistry, Georgia State University, Atlanta, GA 30303.
* Author to whom correspondence should be addressed.

0003-2700/88/0360-2127$01.50/0