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Liquid Chromatographic Resolution of Racemic Drugs on Various α-Arylalkylamine Derived Chiral Stationary Phases

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After conversion to their 3,5-dinitrobenzoyl or 3,5-dinitroanilide derivatives, the enantiomers of a number of drugs may be chromatographically separated on various α-arylalkylamine-derived chiral stationary phases (CSPs). While each CSP used in this study is useful, CSP **1** is best able to resolve the 3,5-dinitroanilide derivative of Ibuprofen while CSP **9** generally gives rather large α values for the resolution of 3,5-dinitrobenzoyl derivatives of the enantiomers of β-adrenergic blocking drugs.

Introduction

Direct chromatographic resolution of enantiomers on chiral stationary phases (CSP's) is now understood as the most rapid, sensitive, accurate and convenient means for determining enantiomeric purity and absolute configuration. In addition, this technique provides the preparative means for obtaining enantiomerically pure samples which may be needed for a variety of purposes. Owing to their potential utility, the development of effective CSPs has challenged a great many workers[1].

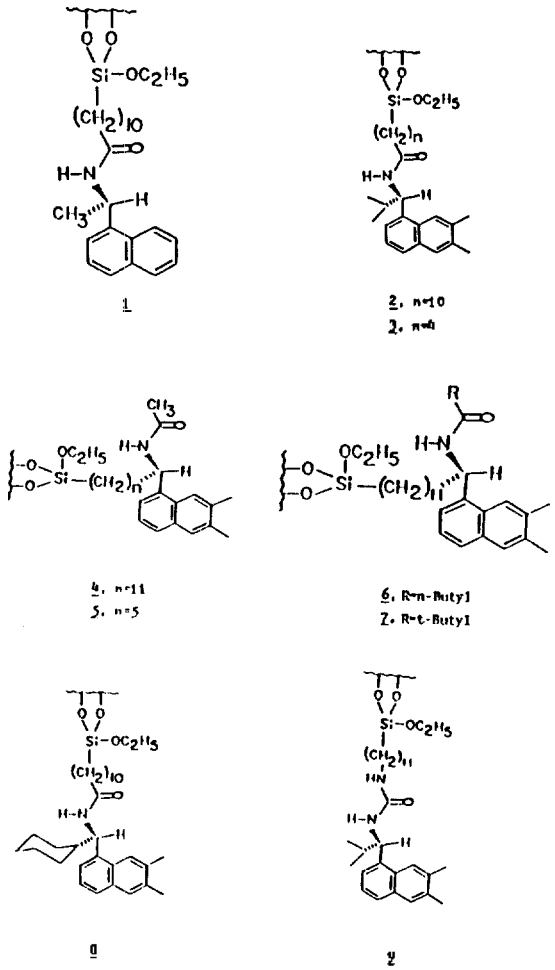
In recent years, our efforts have been focused on the development of new CSPs and the application of these CSPs to the resolution of various racemates. In the course of this work, we prepared α-arylalkylamine derived CSPs **1-9** which prove to be able to resolve the enatiomers of a wide variety of racemates[2-5]. As a general rule, the enantiomers separated by these CSPs must initially be derivatized with an appropriate achiral π-acid reagent owing to the chiral recognition requirements of these CSP.

Among various racemates which are resolvable upon CSP **1-9**, are a number of interesting drugs. It is well known that enantiomers of drugs frequently show quantitatively different pharmacological properties, possibly because one enantiomer better fits the receptor site that does the other[6]. For the study of pharmacokinetics of drug enantiomers, chromatographic determinations of enantiomer concentrations on chiral columns have been suggested as a superior method[7].

We have briefly reported the resolution of several racemic β-adrenergic blocking drugs on CSP **7**[3]. Since each of CSPs **1-9** shows somewhat different abilities to separate drug enantiomers, we now compare the ability of the various α-arylalkylamine-derived CSPs to separate the enantiomers of selected drug derivatives.

Experimental

Preparation of the CSPs used in this study either has been reported[2-5] or will be reported elsewhere. The CSPs were bonded to 5μ Spherisorb and packed into 4.6×250 mm stainless steel columns as methyl alcohol slurries using conventional methods. Chromatography was performed by using an Altex 100 A pump, an Altex 210 injector, an Altex 152 dual wavelength (254 and 280 nm) or an Altex 165 variable wavelength UV detector and a Kipp-Zonen BD 41 recorder. Melting points were taken on a Büchi apparatus and are uncorrected. ^{1}H NMR were recorded on either a Varian EM-390 or Varian XL-200 spectrometer using tetramethylsilane as an internal standard. Infrared spectra were recorded on a Perkin-Elmer 1320 spectrometer. High resolution mass spectra were obtained on a varian 731 mass spectrometer.



All 3,5-dinitrobenzoyl derivatives of racemic drugs used in this study can be prepared using standard Shotten-Bauman procedures. Hence, we report the preparation of but one 3,5-dinitrobenzoyl derivative of a racemic drug as an example of the procedure used.

N-(3,5-Dinitrobenzoyl)propranolol. Propranolol hydrochloride (148 mg, 0.5 mmole) was suspended into 15 m*l* of CH_2Cl_2. To this was added triethylamine (120 mg, 0.17 m*l*, 1.2 mmole) and 3,5-dinitrobenzoyl chloride (110 mg, 0.48 mmole). After stirring for 10 min. at room tempterature, the reaction mixture was washed with 1 N NaOH, 1 N HCl and H_2O. The organic layer was dried over anhydrous Na_2SO_4 and used for HPLC analysis. After purification of the unused portion by preparative TLC, A yellow solid was obtained. mp 161-163°C; ^{1}H NMR($CDCl_3$) δ 1.36 (d, 6H), 3.67-4.00 (m 3H), 4.07-4.50 (m, 4H), 6.73-5.93 (m, 1H), 7.30-7.53 (m, 4H), 7.70-7.85 (m 2H), 8.50-8.55 (m, 2H), 8.90-9.05 (m, 1H). IR (Nujol) 3460, 1625, 1580, 1538 cm^{-1}. High resolution mass spectrum calcd. for $C_{23}H_{23}N_3O_7$: 453.1536, found: 453.1532.

3,5-Dinitroanilide of Ibuprofen. Ibuprofen (206 mg, 1 mmole), thionyl chloride (5 m*l*) and 1 drop of pyridine in a 50 m*l* round bottom flask were refluxed for 1.5 hr with stirring. After removing excess thionyl chloride under vacuo, the residue was dissolved in 20 m*l* of CH_2Cl_2 and then treated with 3,5-dinitroaniline (275 mg, 1.5 mmole) and several drops of triethylamine for 30 min. The reaction mixture was washed

Table 1. Resolution of Racemic Drug Derivatives upon Various α-Arylalkylamine Based Chiral Stationary Phases

Drugs	CSP 1			CSP 2			CSP 3		
	α[a]	k_1'[a,b]	c[c]	α[a]	k_1'[a,b]	c[c]	α[a]	k_1'[a,b]	c[c]
Propranolol[d]	1.10	4.3		1.33	1.3		1.39	1.0	
Propranolol[e]	1.25	14.6	R	1.15	4.8	R	1.27	4.2	R
Alprenolol[e]	1.22	9.9		1.21	2.3		1.21	2.3	
Oxoprenolol[e]	1.20	10.9		1.17	2.5		1.26	2.6	
Metoprolol[e]	1.23	16.0		1.34	5.0		1.82	4.9	
Ephedrin[e]	1.14	13.7		1.98	5.6		3.27	5.1	
Amphetamine[d]	1.11	15.4	R	1.40	9.1	S	1.12	6.5	S
Ibuprofen[f]	3.16	8.5		2.27	2.8		2.95	1.6	

Drugs	CSP 4			CSP 5			CSP 6		
	α[a]	k_1'[a,b]	c[c]	α[a]	k_1'[a,b]	c[c]	α[a]	k_1'[a,b]	c[c]
Propranolol[d]	1.28	2.0		1.28	2.1		1.40	2.0	
Propranolol[e]	1.48	11.0	R	1.40	14.9	R	1.33	9.5	R
Alprenolol[e]	1.39	5.3		1.31	8.4		1.31	5.0	
Oxoprenolol[e]	1.41	5.9		1.41	9.6		1.30	5.5	
Metoprolol[e]	2.26	11.1		2.05	14.4		1.66	10.7	
Ephedrin[e]	2.31	24.4		1.87	19.7		1.69	11.5	
Amphetamine[d]	1.32	12.6	S	1.40	12.6	S	1.45	17.3	S
Ibuprofen[f]	1.72	4.5		1.41	4.9		1.53	8.0	

Drugs	CSP 7			CSP 8			CSP 9		
	α[a]	k_1'[a,b]	c[c]	α[a]	k_1'[a,b]	c[c]	α[a]	k_1'[a,b]	c[c]
Propranolol[d]	1.63	2.1		1.29	1.2		1.53	1.2	
Propranolol[e]	1.24	13.3	R	1.21	3.9	R	2.70	3.6	R
Alprenolol[e]	1.24	7.1		1.23	1.8		1.14	1.8	
Oxoprenolol[e]	1.23	8.0		1.19	2.0		1.18	2.1	
Metoprolol[e]	1.52	14.0		1.43	3.9		1.16	3.9	
Ephedrin[e]	1.48	12.9		2.99	5.0		1.76	4.5	
Amphetamine[d]	1.51	2.1	S	1.35	8.7	S	1.68	9.7	S
Ibuprofen[f]	1.17	11.6		2.80	2.4		1.61	5.8	

[a]See reference 14 for definition. [b]Mobile phase is 20% isopropyl alcohol in *n*-hexane except on CSP 1. Mobile phase for the resolution on CSP 1 is 10% isopropyl alcohol in *n*-hexane. Flow rate is 2 m*l*/min. [c]Absolute configuration of the second eluted enantiomer. For blanks, elution orders have not been established. [d]N-3,5-Dinitrobenzoyl derivative. [e]Bis 3,5-dinitrobenzoyl derivative. [f]3,5-Dinitroanilide derivative.

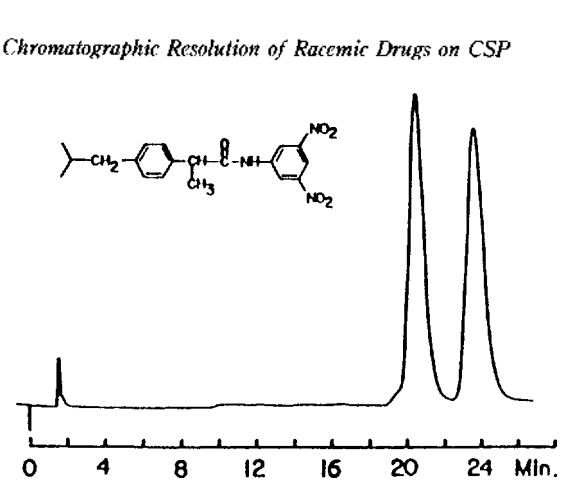


Figure 1. The resulution of 3,5-dinitroanilide derivative of Ibuprofen on CSP **7** using 20% isopropyl alcohol in *n*-hexane as a mobile phase.

with 1 N NaOH, 1 N HCl and H_2O. The organic layer was dried over anhydrous Na_2SO_4 to afford a sample solution for HPLC. Purification by preparative TLC afforded a white solid (304 mg, 82%). mp 194-195°C; ^1H NMR($CDCl_3$) δ 0.90 (d, 6H), 1.58 (d, 3H), 1.80-2.05 (m, 1H), 2.45 (d, 2H), 3.73 (q, 1H), 7.00-7.23 (m, 4H), 7.62 (broad s, 1H), 8.60 (broad s, 3H). IR (Nujol) 3260, 3100, 1670, 1605, 1528 cm^{-1}. High resolution mass spectrum calcd. For $C_{19}H_{21}N_3O_5$: 371.1481, found: 371.1475.

Results and Discussion

Various α-adrenergic blocking drugs, as their N-3,5-dinitrobenzoyl derivatives, resolved quite well on CSPs **1-9**. The N-3,5-Dinitrobenzoyl derivative of the central nervous system stimulant, amphetamine, and the 3,5-dinitroanilide derivative of the antiinflammatory agent, Ibuprofen also resolved well on CSPs **1-9**. Pertinent chromatographic data appear in Table 1 and a chromatogram of the resolution of the Ibuprofen derivative is shown in Figure 1.

As shown in Table 1, each CSP affords characteristic resolution behavior. For example, CSP **1** shows the best result for the resolution of the 3,5-dinitroanilide derivative of Ibuprofen. CSP **3** shows the largest α value for the resolution of the ephedrin derivative. CSP **9** shows the best result for the resolution of the bis-(3,5-dinitrobenzoyl) derivative of Propranolol while CSP **7** shows the best result for the resolution of mono 3,5-dinitrobenzoyl derivative of Propranolol. No chiral recognition mechanism is proposed to account for these differences. Conceivably, there is an averaging between two competing chiral recognition mechansims as has been reported for the resolution of series of N-(3,5-dinitrobenzoyl)-α-arylalkylamines upon α-arylalkylamine derived CSPs[5]. However, we can generally conclude that CSP **7** can be used for the resolution of β-blocking drugs while CSP **1** can be successfully used for the resolution of drugs similar to Ibuprofen. In addition, CSP **1** is quite attractive for the resolution of racemic drugs since it is relatively easy to prepare from the commercially available (R)-1-naphthylethylamine.

Among β-adenergic blockers, racemic Propranolol is the most widely used for the treatment of cardiac dysrhythmias and hypertension. The (S)-enantiomer is known to have 100 times more β-adrenergic blocking activity than does the (R)-enantiomer[8]. Resolution of racemic Propranolol has been achieved by chromatography on silica of the diastereomeric derivatives of either (R)- or (S)-phenylethylisocyanate[9] or (R,R)- or (S,S)-tartaric acid[10]. Propranolol and ephedrin have also been resolved on commercially available chiral N-(3,5-dinitrobenzoyl)-phenylglycine columns as the oxazoline derivatives[11,12]. However, the easy preparation of 3,5-dinitrobenzoyl derivatives of β-adrenergic blocking drugs and the large α values noted on the α-arylaklyamine-derived CSPs make the latter approach particularly attractive. We note that the enantiomers of a number of drugs may be separated *without derivatization* on commerically available immobilized α-glycoprotein columns[13]. Offsetting this advantage, these columns are rather expensive and alledged to sometimes be short-lived.

In conclusion, the easy separation of important racemic drugs upon α-arylalkylamine-derived CSPs may prove useful for pharmacological studies because this technique can be used either preparatively or to moniter the enantiomeric composition of the minute quantities of drugs isolated from whole blood or urine specimens. Such analysis, previously difficult or impossible, is vastly facilitated by chiral chromatographic columns. Procedures for analysis of the enantiomeric composition of β-adrenergic blocking drugs obtained from samples of body fluid are now being developed in our laboratories.

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