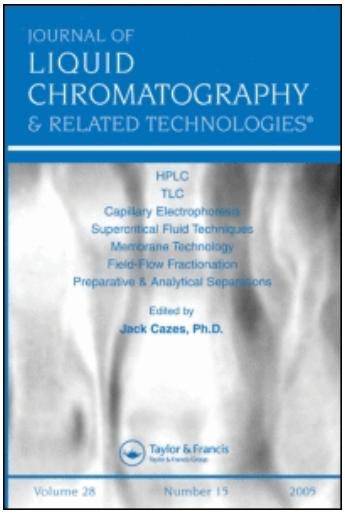

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The Application of HPLC Chiral Stationary Phases to Stereochemical Problems of Pharmaceutical Interest: A General Method for the Resolution of Enantiomeric Amines as β-Naphthylcarbamate Derivatives

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THE APPLICATION OF HPLC CHIRAL STATIONARY PHASES TO STEREOCHEMICAL PROBLEMS OF PHARMACEUTICAL INTEREST: A GENERAL METHOD FOR THE RESOLUTION OF ENANTIOMERIC AMINES AS β-NAPHTHYL-CARBAMATE DERIVATIVES

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ABSTRACT

The enantiomers of primary, secondary and some tertiary amines are resolved as carbamate derivatives formed by reaction with ß-naphthylchloroformate. The enantiomeric carbamates are resolved on a commercially available Pirkle-type HPLC chiral stationary phase (CSP), consisting of (R)-N-(3,5-dinitrobenzoyl)-phenylglycine covalently bonded to silica, by using a mobile phase consisting of mixtures of isopropanol in hexane. In this manner, 12 amines structurally related to amphetamine were resolved, including such pharmacologically important compounds as methamphetamine and pseudoephedrine, neither of which had been previously resolved on Pirkle-type CSPs. Reaction of the haloformate with phenolic amines may be controlled to give either mono- or bis-derivatives; both gave useful resolutions. Simple aliphatic amines such as sec-butylamine were also resolved. The order of elution of the carbamates was consistently R,S; this elution pattern is discussed in terms of specific interactions between the carbamate and the CSP.

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INTRODUCTION

Recent reports from this laboratory (1-6) have shown that the stereochemical composition of pharmaceutical amines may be rapidly and accurately determined by direct enantiomeric resolution on commercially available HPLC chiral stationary phases (CSP). In these studies, the amines were first derivatized with achiral reagents; there are several reasons for this preliminary derivatization step. Free amines generally have poor chromatographic properties on these CSPs; a reaction that forms neutral compounds improves chromatography while at the same time allowing the incorporation of functional groups which may enhance enantiomeric selectivity on the CSP and also confer or amplify ultraviolet or fluorescence detectability.

However, some limitations restrict the scope of these procedures. For example, although the enantiomers of the primary amine amphetamine were resolved as any of a number of amide derivatives (1), the secondary amine analogue methamphetamine was not resolved by this procedure. Ephedrine, a 1,2-aminoalcohol, was resolved following condensation with various aromatic aldehydes to form cyclic oxazolidines (3,4). Separation factors (α) as high as 1.36 were observed, yet none of the oxazolidines of pseudoephedrine (the diastereomer of ephedrine) gave detectable resolution. Other 1,2-aminoalcohols such as norephedrine (5) and propranolol (6) were condensed with phosgene, and the enantiomers were chromatographed as the cyclic

oxazolidones. This reaction does not add a chromophore to the molecule and thus does not enhance the detectability of the amine, thereby limiting its usefulness in pharmacological studies of compounds such as norephedrine, which has an intrinsically low electronic absorptivity.

The well-known reaction of amines with haloformates affords carbamates, which have been shown to have chromatographically useful properties in achiral systems (7,8). The reaction is general for primary and secondary amines (9), as well as for some tertiary amines, which are selectively cleaved at the nitrogen atom (7,8). Reaction with difunctional compounds such as hydroxylic and phenolic amines can be precisely controlled to give well-defined products (10).

The chromatography of carbamates on CSPs has not been extensively studied, although resolution of some individual cyclic carbamates (oxazolidones) has been incidentally reported (5,6), and some carbamates prepared from aliphatic chloroformates also have been resolved (11). In this paper we describe a general method for the resolution of enantiomeric amines as carbamate derivatives formed by reaction with ß-naphthylchloroformate. The enantiomers are resolved on a commercially available Pirkle-type covalent (R)-N-3,5-(dinitrobenzoyl)phenylglycine CSP by using mixtures of isopropanol:hexane as the mobile phase. The scope of the method is illustrated by results for a series of 15 amines of

pharmaceutical interest; these compounds, which are structurally related to amphetamine, were chosen for their functional diversity. Twelve of these compounds were resolved, including such classically simple molecules as sec-butylamine.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 3500 liquid chromatograph equipped with a 10 or 100 µl sample loop, an SP Model 8200 single wavelength UV/VIS detector with a 254 nm lamp and an SP Model 4000 data system. The column was a 250 X 4.6 mm id or a 250 X 10 mm id Regis (Morton Grove, IL, U.S.A.) stainless steel Pirkle covalent type, packed with 5 µm spherical γ-aminopropyl silica particles modified with (R)-N-(3,5-dinitrobenzoyl)phenylglycine.

Mass spectra were obtained with a Finnigan MAT (San Jose, CA, U.S.A.) 4023-T gas chromatograph-mass spectrometer.

Chemicals

Isopropanol and hexane were HPLC grade. All other salts, solvents and reagents were reagent grade and were used as obtained. Phosgene was obtained as a solution in toluene (Aldrich, Milwaukee, WI, U.S.A.).

The racemic amines were readily available, in the form of salts or free amines, as pharmaceutical reference standards or from commercial sources, except that dl-norpseudoephedrine (as a mixture with dl-norephedrine) was a gift from Loren Gelber (FDA Regional Laboratory, Winchester, MA, U.S.A.). Enantiomerically pure samples were from the following sources: d- and l-pseudoephedrine base and d-norpseudoephedrine base from Sigma (St. Louis, MO, U.S.A.); d-ephedrine HCl and l-ephedrine nitrate from Aldrich (Milwaukee, WI, U.S.A.); d-amphetamine sulfate and d- and l-p-hydroxyamphetamine HBr from Smith Kline and French (Philadelphia, PA, U.S.A.); d-benzphetamine HCl from Upjohn (Kalamazoo, MI, U.S.A.); and d-methamphetamine HCl from U.S. Pharmacopeia (Rockville, MD, U.S.A.). d-Norephedrine was a research material prepared in this laboratory (5).

Synthesis of ß-Naphthylchloroformate

This method is a modification of that described by Moszczynski et al. (12). Cold 12.5% $COCl_2$ in toluene (550 ml, 0.694 mol) was added dropwise with mechanical stirring to 100 ml of an aqueous solution of ß-naphthol (35 g, 0.243 mol) and NaOH (15 g, 0.375 mol). This solution was cooled with an ice/salt bath. After addition of reagent, the solution was stirred for 2 h; then excess $COCl_2$ was removed under vacuum by using a gas bubbler containing a 40% aqueous solution of NaOH as a trap. After addition of 100 ml H_2O, the phases separated; the organic

phase was collected, washed with 10% NaOH solution and then with H_2O and dried with Na_2SO_4. The toluene was then removed under vacuum (35°C at 50 mm Hg). The residue was extracted with ether, and the ethereal solution was filtered and evaporated to yield 40 g of an orange-white solid (80% crude yield). The crude product was refrigerated under N_2 until needed.

To maintain a reagent of high purity for the derivatization reaction, it was desirable to purify the reagent in small portions and use it within a few days. Purification was accomplished by placing a 2 g portion of crude product dissolved in 0.5 ml CH_2Cl_2 atop a 2 cm (id) glass column packed with a hexane slurry of 15 g silica gel (60-200 mesh) and then eluting with 200 ml hexane. Unreacted naphthol and air oxidation products (naphthoquinones) are strongly retained. Evaporation of the hexane eluate produced white crystalline ß-naphthylchloroformate (mp 56-58°C) in 60-65% yield (based on crude product). The purified reagent was kept refrigerated under N_2 and shielded from light until it was used.

Preparation of Carbamates

Typically, 2-50 mg of the amine or amine salt was derivatized by using a 2- to 10-fold molar excess of ß-naphthylchloroformate and a total reaction volume of approximately 20 ml. Four distinct techniques were employed:

(a) Amine free base and haloformate reagent were each dissolved in an organic solvent (ether or methylene chloride);

the solutions were mixed, and an excess of triethylamine was optionally added to consume HCl (otherwise yields are diminished by precipitation of amine HCl); the reaction mixture was stirred at room temperature for 5 min.

(b) A freshly prepared solution of haloformate reagent in a mixture of 1:1 pH 7 phosphate buffer:dioxane was added to an amine salt dissolved in the same solvent. After the reaction had proceeded for 5 min at room temperature, the product was extracted with an equal volume of methylene chloride.

(c) A solution of the amine salt in pH 7 phosphate buffer was shaken for 5 min with a solution of the haloformate in an equal volume of methylene chloride.

(d) For bis-derivatives of phenolic amines, a solution of the amine salt in pH 10 carbonate buffer was shaken for 5 min with an equal volume of a methylene chloride solution containing the haloformate and 10 mg tetrapentylammonium bromide.

In each of the above procedures the organic phase was optionally washed with an aqueous alkaline solution to remove naphthol, then washed with H_2O and dried over Na_2SO_4 before chromatography.

Chromatographic Conditions

The mobile phase was either 5 or 20% isopropanol in hexane, at flow rates of 2 and 10 ml/min for the analytical and preparative columns, respectively. Before injection, each solution to be analyzed was filtered through a Millex-SR 0.5 μm

PTFE disposable membrane filter (Millipore, Bedford, MA, U.S.A.). The temperature of the system was maintained at 25°C.

RESULTS AND DISCUSSION

Preparation and Identification of the Carbamates

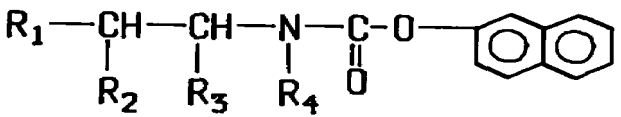
The ß-naphthylchloroformate reagent was synthesized from ß-naphthol and phosgene according to the literature (12). In addition, a final purification by chromatography on silica was performed. The reagent is stable indefinitely when refrigerated.

The reaction of the haloformate with both primary and secondary amine functions is essentially instantaneous and quantitative at room temperature. Carbamates which were prepared in this study are listed in Table 1. A variety of preparative strategies were investigated (see EXPERIMENTAL); these included both aqueous and nonaqueous one-phase systems, and two-phase systems with and without ion-pair catalysis. All of these approaches were successful; the choice depends in practice on the source and solubility properties of the analyte, and on the number and nature of functionalities present. All of these methods maintain the original enantiomeric composition, since the amine analyte is the only chiral component of the reaction mixture, and no racemization occurs under the mild conditions employed.

Tertiary amines can also be derivatized by using the ß-naphthylchloroformate reagent. For example, the tertiary amine

TABLE 1

Structures and Chromatographic Results for the Carbamate Derivatives



No.	Derivatized Compound	R_1	R_2	R_3	R_4	i-PrOH, %	k'1	k'2	α	Elution Order[a]
1	Amphetamine	Ph	H	Me	H	5	9.79	11.02	1.13	S
2	Methamphetamine	Ph	H	Me	Me	5	7.51	8.53	1.14	S
3	Benzphetamine[b]	Ph	H	Me	Me	5	7.51	8.53	1.14	S
4	Methoxyphenamine	m-MeO-Ph	H	Me	Me	5	7.91	9.29	1.17	--[c]
5	Norephedrine	Ph	OH	Me	H	5	15.81	16.57	1.06	1R, 2S
6	Norpseudoephedrine	Ph	OH	Me	H	5	22.38	24.60	1.10	1S, 2S
7	Ephedrine	Ph	OH	Me	Me	5	14.92	15.57	1.04	1R, 2S
8	Pseudoephedrine	Ph	OH	Me	Me	5	17.24	20.57	1.22	1S, 2S
9	p-Methoxyephedrine	p-MeO-Ph	OH	Me	Me	5	11.69	--	1.00	--
10	Halostachine	Ph	OH	H	Me	5	12.02	--	1.00	--
11a	p-Hydroxyamphetamine	p-OH-Ph	H	Me	H	20	8.49	8.99	1.06	S
11b	" (bis-derivative)	p-OH-Ph	H	Me	H	20	17.94	20.73	1.16	S
12a	p-Hydroxyephedrine	p-OH-Ph	OH	Me	Me	20	11.10	--	1.00	--
12b	" (bis-derivative)	p-OH-Ph	OH	Me	Me	20	22.77	25.54	1.12	--[c]
13a	Phenylephrine	m-OH-Ph	OH	H	Me	20	13.57	--	1.00	--
13b	" (bis-derivative)	m-OH-Ph	OH	H	Me	20	33.20	--	1.00	--
14	sec-Butylamine	Me	H	Me	H	5	7.22	7.66	1.06	--[c]
15	2-Aminoheptane	n-Pentyl	H	Me	H	5	4.95	5.22	1.17	--[c]

[a]Configuration of second eluted isomer.
[b]Tertiary amine: N-benzyl-N-methyl-1-phenyl-2-aminopropane; the elution order was assigned by analogy with the results for 2.
[c]Not determined.

benzphetamine (N-benzyl-N-methyl-1-phenyl-2-aminopropane, 3) was cleaved by reaction of the free amine with haloformate in ether at room temperature to afford a carbamate identical to that from methamphetamine, 2. Cleavage of tertiary amines by arylhaloformates is well established as a prechromatographic derivatization technique (7,8); the facile loss of the N-benzyl group is likewise in accord with the known lability of this group under the conditions of this reaction (13). Many chiral tertiary amine pharmaceuticals are potential candidates for similar cleavage, although more forcing conditions may be required (8).

The reaction of the three phenolic amines in this study (11, 12 and 13) with ß-naphthylchloroformate was readily controlled to selectively involve only the amino group (mono-derivative) or both the amine and phenolic functions (bis-derivative) as shown in Figure 1. The course of this two-stage reaction has been studied for similar compounds (10). The bis-derivative was obtained in a two-phase system containing added tetraalkylammonium cations; it is probable that the reacting species is the phenolate moiety of the extracted ion-pair; the oxygen atom in this form is sufficiently nucleophilic to attack the carbonyl function of the haloformate reagent.

In general, the carbamates were not routinely isolated but were chromatographed directly, with little or no cleanup, as solutes in organic solvents. However, representative carbamate eluates were individually collected from a semipreparative

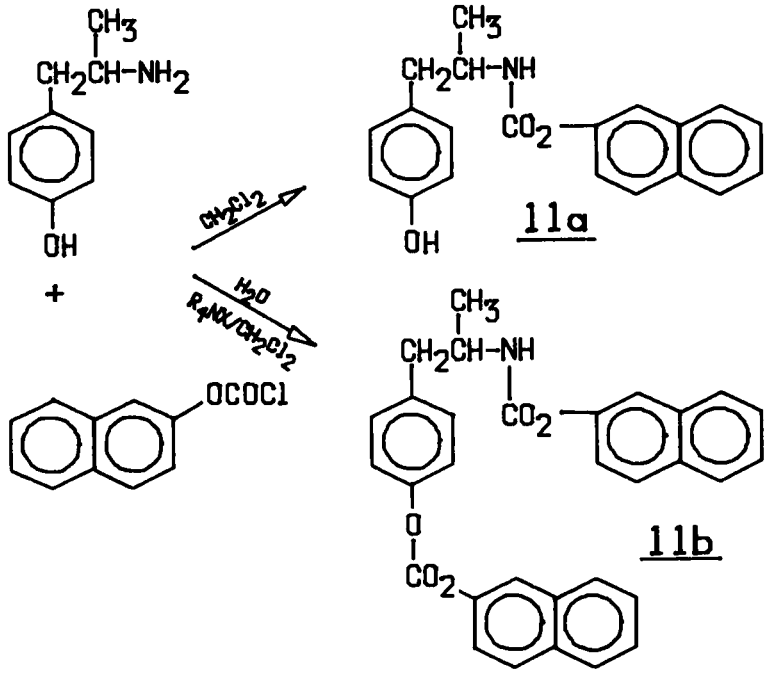


FIGURE 1. The reaction of p-hydroxyamphetamine with ß-naphthylchloroformate to give the N-ß-naphthyl derivative, 11a, and the N,O-bis-(ß-naphthyl)derivative, 11b; see EXPERIMENTAL for reaction condtions.

phenylglycine CSP column, evaporated to a colorless oil and identified by mass spectrometry. In this manner, the structures of carbamates of the following compounds were identified by their molecular ions and major fragmentation ions: amphetamine, 1; methamphetamine, 2; methoxyphenamine, 4; ephedrine, 7; and the mono-derivative of p-hydroxyamphetamine, 11a. In particular, it was shown that the aliphatic hydroxyl group of ephedrine does not react with the haloformate reagent under the conditions employed.

We were not able to obtain the mass spectrum of the bis-derivative of p-hydroxyamphetamine, 11b, because it decomposed under the conditions of collection.

As proof of enantiomeric resolution, in the case of the carbamate of methoxyphenamine, 4, the eluates containing the individual enantiomers were collected for mass spectrometric analysis; the two mass spectra were identical in every respect.

Chromatography

The chromatographic results are presented in Table 1. The CSP was the covalent modification of (R)-N-(3,5-dinitrobenzoyl)-phenylglycine (14,15). The mobile phase was 5% isopropanol in hexane, except for the chromatography of the phenolic amines, which required 20% isopropanol in hexane for satisfactory capacity factors.

Twelve of the 15 amines were resolved. In contrast to chromatographic results for other derivatives, carbamates derived from secondary amines were resolved about as well as or better than those from the corresponding primary amines. Three direct comparisons are given in Table 1: 1:2, 5:7 and 6:8. Capacity factors for the more lipophilic secondary amines are consistently lower, as expected.

The results in Table 1 do not necessarily represent the optimum chromatographic conditions; in particular, addition of small amounts of acetonitrile to the mobile phase improves column

efficiency with minimal effect on separation factors. Typical optimized separations are shown in Figure 2 for methamphetamine and for pseudoephedrine. These pharmacologically important secondary amines have not previously been resolved on commercially available CSPs.

Incorporation of a naphthyl function in the molecule serves two purposes. Because of its π-basic properties, the naphthyl group can interact strongly with the π-acidic 3,5-dinitrobenzoyl group of Pirkle-type CSPs; this interaction enhances enantiomeric resolution. In addition, the naphthyl chromophore is highly sensitive to both ultraviolet and fluorescence detectors.

Enantiomeric elution orders were obtained for eight of the carbamates by injecting known, unequal mixtures of the enantiomers. In every case so far determined, the (S)-isomer is best retained (second eluted). This is the same order as that which we previously observed (1), with one exception (16), for amides of amphetamine. However, in view of a growing appreciation among workers in the field concerning the complexities and uncertainties involved in chiral recognition, especially for conformationally nonrigid systems, we refrain at present from offering a detailed interaction model.

Nevertheless, we make some observations based on the present results which may contribute to an understanding of recognition processes on Pirkle-type CSPs.

(a) For the diastereomeric pairs ephedrine:pseudoephedrine and norephedrine:norpseudoephedrine, it is the chirality at C-2

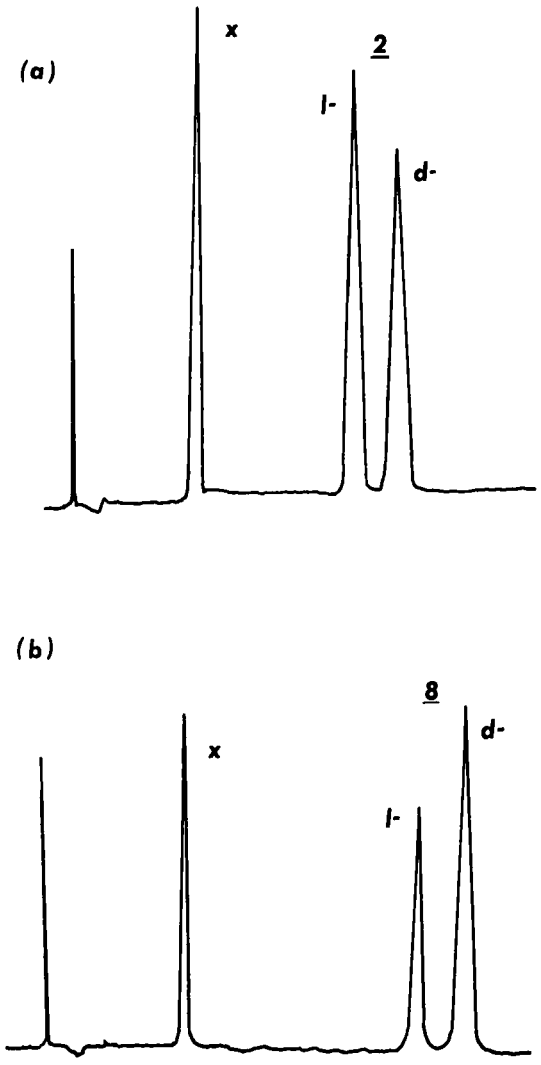


FIGURE 2. Chromatograms representing optimal enantiomeric resolutions of the carbamates of (a) a 50:50 mixture of d- and l-methamphetamine, 2; the mobile phase was hexane:isopropanol:acetonitrile 99:0.5:0.5, and (b) a 60:40 mixture of d- and l-pseudoephedrine, 8; the mobile phase was hexane:isopropanol:acetonitrile 95:4:1. The chart expansion was different for the two chromatograms; the peak marked x is present in most chromatograms and is tentatively attributed to bis-ß-naphthylcarbonate.

(the nitrogen-substituted carbon) rather than at C-1 (the hydroxyl-substituted carbon) that determines the elution order. In all four compounds, the (2S)-isomer is best retained.

(b) This is reinforced by the fact that two of the three amines which were not resolved (halostachine, 10, and phenylephrine, 13) lack a chiral center at the amino-substituted carbon. (This also appears to represent a practical limitation on the scope of the proposed method.)

(c) The third compound which was not resolved, p-methoxyephedrine, 9, contains a π-basic aromatic substituent which may compete with the introduced naphthyl group for π-π interaction with the π-acidic dinitrophenyl group of the CSP (17).

(d) The importance of π-π interactions in the recognition process is also demonstrated by the lack of resolution, in limited trials, for chiral phenyl and p-nitrophenyl carbamate derivatives of amphetamine.

(e) The covalent leucine modification of this CSP has so far failed to resolve any of the carbamate derivatives in the amphetamine series, i.e., compounds where R_1 is phenyl or substituted phenyl. This suggests the possibility of a second π-π interaction involving R_1 and the phenyl ring of the phenylglycine moiety of the CSP, as we have proposed previously for amide derivatives (1).

(f) This idea is reinforced by the consistently greater resolution of the bis-derivatives of the phenolic amines,

compared to that of the mono-derivatives. The bis-derivatives have two naphthyl rings available for π-π interaction.

(g) Finally, the resolution of the carbamates of the aliphatic amines sec-butylamine, 14, and 2-aminoheptane, 15, are especially significant in terms of understanding the mechanism(s) of chiral recognition. All of the potential bonding interaction sites of these simple carbamates are joined to the chiral carbon through the same N-C bond. This bond is, therefore, free to rotate within the solute:CSP complex, with rotational populations controlled only by steric repulsions involving the other three groups as they interact with the CSP and with the remainder of the solute molecule. Thus, the resolutions of these simple compounds are impressive; elucidation of the mode of chiral recognition for such structures constitutes an important challenge for theories of chiral chromatography.

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