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APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC CHIRAL STATIONARY PHASES TO PHARMACEUTICAL ANALYSIS: STRUCTURAL AND CONFORMATIONAL EFFECTS IN THE DIRECT ENANTIOMERIC RESOLUTION OF α-METHYLARYLACETIC ACID ANTI-INFLAMMATORY AGENTS

IRVING W. WAINER* and THOMAS D. DOYLE
Division of Drug Chemistry, Food and Drug Administration, Washington, DC 20204 (U.S.A.)
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SUMMARY

A series of four α-methylarylacetic acids of pharmaceutical interest were directly resolved as enantiomeric amide derivatives on a high-performance liquid chromatographic chiral stationary phase consisting of covalently bound (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine. Enantiomers of ibuprofen, naproxen, fenoprofen and benoxaprofen all exhibited optimum separation (α = 1.12, 1.23, 1.12 and 1.10, respectively) as 1-naphthalenemethylamides. Derivatives of ibuprofen were studied in detail; measurable separations were observed for secondary and tertiary amides of diverse structural types, but not for the primary amide or for ester derivatives. The results, which are consistent with an interaction model involving stacking of amide dipoles, with or without supplementary π–π and hydrogen-bonding effects, appear to be strongly dependent on conformational requirements of both the solute and the chiral stationary phase.

INTRODUCTION

Although the high-performance liquid chromatographic (HPLC) chiral stationary phases (CSPs) developed by Pirkle *et al.*[1–3] have been successful in the direct enantiomeric resolution of a variety of chiral molecules, not all structural types or functionalities are equally resolvable. For example, both amines and carboxylic acids are typically difficult to resolve, whereas simple amide derivatives are often the most successfully resolved. We have shown that compounds of the first type, chiral amines of pharmaceutical interest such as amphetamine, can be readily resolved by preparation of a variety of amide derivatives[4,5]. Pirkle *et al.* have studied compounds of the second type and have reported the separation of primary amides of a number of α-substituted phenylacetic acids[2]. This paper reports the expansion of this approach to a series of α-methylarylacetic acid anti-inflammatory agents that are resolved as secondary and tertiary amides.

A number of α-methylarylacetic acid anti-inflammatory agents are widely used

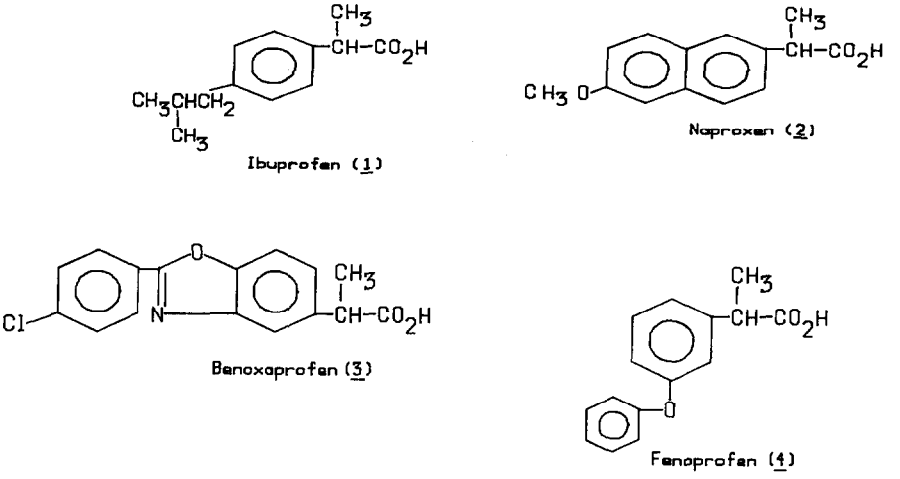

Fig. 1. Structures of ani-inflammatory agents used in this study.

for the relief of acute and chronic rheumatoid arthritis and osteoarthritis. Three members of this class shown in Fig. 1, ibuprofen (1), naproxen (2) and fenoprofen (4), are respectively the 8th, 23rd and 99th most frequently prescribed drugs in the United States[6]. A fourth drug, benoxaprofen (3) (Fig. 1), is currently available only on a limited basis. Of the four, only naproxen is administered as the resolved (*S*)-(+)-enantiomer.

The resolution of these molecules is of pharmacological interest because of the fact that the (*R*)-(−)-enantiomers of ibuprofen[7], naproxen[8] and benoxaprofen[9] were found to be converted *in vivo* into the corresponding (*S*)-(+)-enantiomers. In each case, the enantiomeric compositions were determined by conversion of the enantiomeric acids into diastereoisomeric amides, which were then separated by gas–liquid chromatography (GLC)[7,9] or HPLC[8].

Although the diastereoisomeric approach to analysis commonly provides excellent chromatographic separations, it contains the inherent risk of undetected bias of the results because of partial racemization of the derivatizing reagent or unequal reaction rates. By contrast, the direct enantiomeric approach described in this report uses achiral reagents, thus avoiding these pitfalls.

EXPERIMENTAL

Apparatus

The chromatography was performed with a Spectra-Physics (Santa Clara, CA, U.S.A.) Model 8000 liquid chromatograph equipped with an SP 8000 data system, a Spectra-Physics Model 8310 UV–visible detector set at 254 nm and a temperature-controlled column compartment. The column was a stainless-steel J. T. Baker-packed Pirkle covalent column (25 cm × 4.6 mm I.D.) with an α-aminopropyl packing of 5-μm spherical particles modified with (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine (J. T. Baker, Phillipsburg, NJ, U.S.A.). ^{1}H Nuclear magnetic resonance (NMR) spectra were obtained with a 200-MHz Fourier transform NMR spectrometer (Varian XL-

200, Varian Assoc., Instrument Group, Palo Alto, CA, U.S.A.). Optical rotations were measured with a Model 241MC polarimeter (Perkin-Elmer, Norwalk, CT, U.S.A.).

Materials

Thionyl and oxalyl chlorides, 1-naphthalenemethylamine and the other amines used in this study were purchased from Aldrich (Milwaukee, WI, U.S.A.). The racemic anti-inflammatory agents were obtained from their respective manufacturers and (*R*)-(−)- and (*S*)-(+)-naproxen were supplied by Syntex Labs. (Palo Alto, CA, U.S.A.). The HPLC solvents were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). The remaining chemicals and solvents were reagent grade and were used as purchased.

Amide synthesis using thionyl chloride

The amides of ibuprofen, fenoprofen and benoxaprofen were synthesized by first converting the acid into the acid chloride with thionyl chloride and following this reaction by the addition of the appropriate amine.

In a typical run, ibuprofen (2.06 g, 0.01 mole) was refluxed for 1 h with 0.6 ml of thionyl chloride. The excess thionyl chloride was removed under reduced pressure. The resulting solid was dissolved in 15 ml of chloroform, and 1-naphthalenemethylamine (1.57 g, 0.01 mole) was added dropwise; this mixture was stirred for 1 h. The chloroform layer was then washed three times with 4 *N* hydrochloric acid and dried over sodium sulfate. Evaporation of the solvent yielded a yellow solid, which was recrystallized from ethanol–water to produce a colorless crystalline material. The desired amide was obtained in 74% yield.

Amide synthesis using oxalyl chloride

The amides of naproxen were synthesized by converting the acid into the acid chloride with oxalyl chloride and following this reaction by the addition of the appropriate amine. The method is a modification of the procedure described by Lan *et al.*[10].

In a typical run, naproxen (0.05 g, 0.002 mole) was refluxed for 15 min with 5.0 ml of oxalyl chloride. The solution was evaporated to dryness under a stream of nitrogen. The resulting solid was dissolved in 5.0 ml of chloroform and 1-naphthalenemethylamine (0.31 g, 0.002 mole) was added dropwise; this mixture was stirred for 1 h and then extracted three times with 4 *N* hydrochloric acid. The chloroform layer was dried over sodium sulfate and evaporated, giving the desired product in 88% yield after recrystallization from ethanol.

Ester synthesis using thionyl chloride

Esters of ibuprofen, compounds 1h–1j in Table I, were synthesized by using the acid chloride and the appropriate alcohol. The acid chloride, which was synthesized as described above, was then dissolved in chloroform and an equimolar amount of the alcohol and a few drops of pyridine were added. The solution was allowed to stand for 1 h at room temperature. Reaction products 1h and 1j were recrystallized from ethanol–water; reaction product 1i, an oil, was purified by HPLC on silica gel.

TABLE I

CHROMATOGRAPHIC RESULTS

Compound	k_1'*	α	R_s	*Mobile phase***	*First eluted enantiomer*
1 Ibuprofen					
1a Primary amide	12.3	1.00	0.00	99:1	
1b N-Methylamide	6.1	1.05	0.58	97:3	(*S*)
1c N,N-Dimethylamide	4.5	1.08	0.68	99:1	(*S*)
1d Cyclohexylamide	11.1	1.04	0.56	99:1	(*S*)
1e Benzylamide	12.0	1.11	1.37	99:1	(*S*)
1f N-Methylbenzylamide	6.4	1.07	0.89	99:1	(*S*)
1g 1-Naphthalenemethylamide	15.5	1.12	1.75	97:3	(*S*)
1h Benzyl ester	1.5	1.00	0.00	99:1	
1i 1-Naphthalenemethyl ester	2.1	1.00	0.00	99:1	
1j 9-Anthracenemethyl ester	2.8	1.00	0.00	99:1	
2 Naproxen					
2a Cyclohexylamide	11.3	1.00	0.00	97:3	
2b Benzylamide	6.9	1.18	1.55	97:3	(*S*)
2c 1-Naphthalenemethylamide	11.7	1.23	1.94	97:3	(*S*)
3 Benoxaprofen					
3a 1-Naphthalenemethylamide	24.2	1.12	1.22	97:3	
4 Fenoprofen					
4a 1-Naphthalenemethylamide	12.2	1.10	0.98	97:3	

* Capacity factor of the first eluted enantiomer.

** Hexane–isopropanol; all compounds were eluted at a flow-rate of 2 ml/min and a temperature of 20°C.

Resolution of ibuprofen

Ibuprofen was partially resolved as the α-methylbenzylamine salt according to the procedure described by Kaiser *et al.*[7].

RESULTS

A series of seven amides of ibuprofen were synthesized and the products were unambiguously identified through their NMR and IR spectra. Table I lists the chromatographic results obtained with the CSP for these amides, compounds 1a–1g. A measurable separation was achieved for all the secondary and tertiary amides of ibuprofen, but not for the primary amide. The best resolutions were obtained for the secondary amides with aromatic substituents, *i.e.*, benzyl and 1-naphthalenemethyl groups (see, *e.g.*, Fig. 2), whereas the resolution of the tertiary N-methylbenzylamide was no better than that of the secondary and tertiary aliphatic amides.

To determine whether racemization occurs during the derivatization process and to elucidate the elution order of the enantiomers, the enantiomers of ibuprofen were partially resolved as their (*R*)-(+)-methylbenzylamide salts according to the procedure described by Kaiser *et al.*[7]. The specific rotation ($[\alpha]_D^{25}$) of the regenerated acid was −48°. The chromatogram of the 1-naphthalenemethylamide of this acid contained two peaks with the same capacity factors as the corresponding peaks from

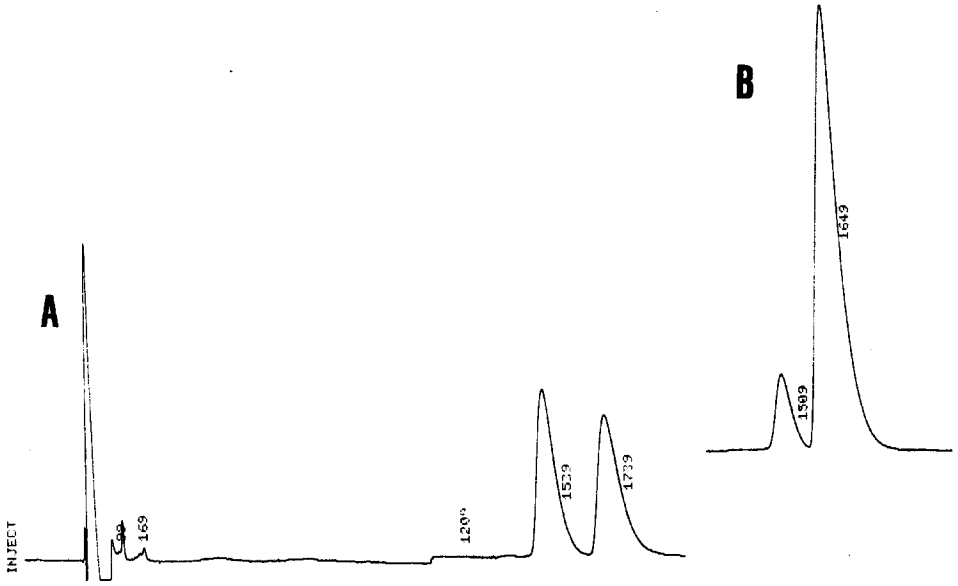

Fig. 2. Resolution of enantiomeric mixtures of ibuprofen 1-naphthalenemethylamide (compound 1g). (A) Racemic mixture; (B) 9:91 mixture of the (*S*)- and (*R*)-enantiomers.

the racemic mixture, but in a ratio of 9:91. This result is in agreement with a report[7] that an observed specific rotation, $[\alpha]_D^{25} = -57°$, corresponds to a 4:96 enantiomeric mixture of the (*S*)-(+)- and (*R*)-(−)-enantiomers.

The partially resolved ibuprofen prepared above was derivatized with a series of amines to produce amides 1b–1g in Table I. In each case, the observed enantiomeric ratio of the products was again 9:91. On the basis of the results, it was determined that the derivatization procedure using thionyl chloride proceeds without racemization. The mixture was also used to determine the elution order of the enantiomers, and in each case the (*S*)-(+)-enantiomer eluted before the (*R*)-(−)-enantiomer (Table I).

Three esters of ibuprofen, compounds 1h–1j in Table I, were also synthesized from the acid chloride with benzyl, 1-naphthalenemethyl and 9-anthracenemethyl alcohols. These esters were not retained on the column (k'_1 ranged from 1.5 to 2.8) and were not resolved.

On the basis of the results with ibuprofen, 1-naphthalenemethylamine was chosen as the derivatizing agent for the other anti-inflammatory agents. Reaction of naproxen (2) with thionyl chloride produced a dark viscous oil which was discarded. The material was treated instead with oxalyl chloride, which yielded the desired acid chloride. The reaction of the acid chloride with cyclohexyl, benzyl and 1-naphthalenemethyl amines produced amides 2a, 2b and 2c (Table I), respectively. The (*R*)-(−)- and (*S*)-(+)-enantiomers of naproxen were allowed to react separately; each amide produced a single chromatographic peak, demonstrating that the derivatization proceeds without racemization. The (*S*)-(+)- and (*R*)-(−)-enantiomers in a 25:75 mixture were converted into the amides and chromatographed; from the chromatographic separations it was determined that the (*S*)-(+)-enantiomer elutes before

the (*R*)-(−)-enantiomer for the benzyl and the 1-naphthalenemethyl amides. It is of interest that the cyclohexylamide, 2d, was not resolved under these conditions.

The results obtained with fenoprofen (3) and benoxaprofen (4) are also reported in Table I for amides 3a and 4a, respectively. In these cases, thionyl chloride was used to convert the free acid into the corresponding acid chloride. These materials were not resolved before chromatography; therefore, the enantiomeric elution order was not determined.

DISCUSSION

The CSP employed in this study was (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine, covalently bonded via an amide linkage between the carboxyl group of the phenylglycine and the α-aminopropyl groups of the support. This CSP possesses a large number of sites which can, in principle, interact with solute molecules in various combinations and in various conformations of both CSP and solute to give a still larger number of conceivable interaction modes. For a given interaction mode to produce a chromatographically measurable distinction between enantiomers, this mode must involve considerable constraints on the spatial orientation of solute to CSP so that stereochemical differences between enantiomeric solute molecules are meaningful.

Dalgliesh[11] has proposed a three-point interaction model as a prerequisite for achieving the necessary constraints; however, he considered only the simple case where each point of interaction involves a discrete bond to the asymmetric center. In the case of the amides in this and previous studies[3–5], two or more of the potentially strongest interaction sites are located on the same bond leading to the asymmetric center. For enantiomeric discrimination to be observed in this situation, it is also necessary that there be constraints on conformational freedom (pre-existing or as a result of interaction) for both solute and CSP.

These considerations lead to an interaction model for the amides in this study which satisfactorily accounts not only for the relative separation of the amide derivatives but also for the observed elution order. This model is similar in most respects to that proposed by Pirkle *et al.*[2,3] in which chiral recognition for amides arises largely from CSP–solute complexes formed primarily by amide dipole stacking between the CSP and solute.

In the present case, molecular models show that maximum alignment of dipoles with the simultaneous optimal approach of the aryl group of the solute to the 3,5-dinitrobenzoyl group of the CSP occurs only in a single conformation of the solute. In this conformation, the α-methyl group (directly attached to the asymmetric carbon) is situated in an unhindered position on top of the solute-CSP complex only in the case of the (*R*)-isomer (Fig. 3). In the (*S*)-isomer, this methyl group sterically interferes with optimum interaction. This explanation is consistent with the observed elution order. The conformation depicted for the CSP in Fig. 3 is that which Pirkle *et al.*[2] have previously shown to be the most stable for this molecule.

The well-known ability of amides to associate through dipole–dipole interactions[12] is a reasonable explanation of the observed resolutions of both the secondary and tertiary amides. The resolution observed for the N-methyl (1b) and the N,N-dimethyl (1c) amides, $\alpha = 1.05$ and 1.08, respectively, requires that a mechanism

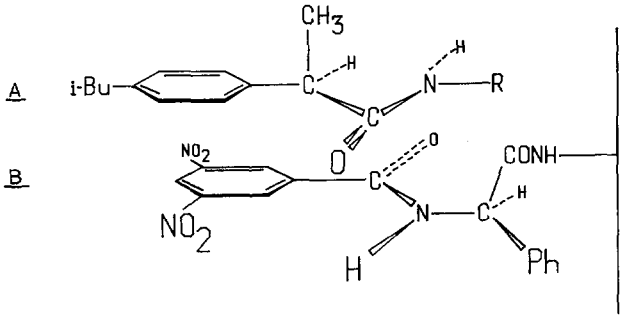

Fig. 3. Postulated orientation and conformations for optimum interaction between A, the amide derivatives of (*R*)-ibuprofen and B, the CSP. Note the stacking of amide dipoles, the proximity of the aromatic rings and the sterically unstrained location of the methyl group attached to the asymmetric carbon of A.

other than hydrogen bonding must be operating as the key regulator in the formation of the CSP–solute complex.

In the case of the ibuprofen amides, therefore, it appears that the driving force behind the formation of the CSP–solute complex is dipole–dipole stacking between the amide function in the CSP and the amide moiety in the solute. This complex is further stabilized as the solute assumes the conformation which maximizes the π–π interaction between the dinitrobenzoyl moiety on the CSP and the aromatic portion of the ibuprofen molecule. Hydrogen-bonding interactions or additional π–π interactions can result in further stabilization. Chiral discrimination is then a function of steric interactions in the solute–CSP complex arising from the α-methyl group on the asymmetric carbon.

This model is supported by a number of experimental observations, including the resolution of both secondary and tertiary amides. An added stability arising from the availability of hydrogen bonding is suggested by the difference in resolution between the N-methylbenzyl (1f) and benzyl (1e) amides. The contributions of the additional π–π interaction site are also suggested by the enhanced resolution of the benzyl (1e) and 1-naphthalenemethyl (1g) amides.

The model also illustrates why there is no reversal of elution order when the 1-naphthalenemethyl moiety is added to the molecule. If π–π interactions were the primary driving force in the formation of the CSP–solute complex, a reversal in elution order of the ibuprofen enantiomers would be expected with the addition of the 1-naphthalenemethyl group. This reversal would reflect the preferred π–π bonding between the naphthyl ring and the 3,5-dinitrobenzoyl ring. In the case of naproxen, the competition between the two naphthyl moieties should lead to a reduction in the observed resolution. The fact that neither of these changes takes place is consistent with a model based on the primacy of the dipole–dipole interaction.

The inability of this CSP to resolve the esters of ibuprofen further supports this model. The dipole moments of esters formed between saturated monohydroxylic alcohols and saturated monocarboxylic acids are in the range 1.7–2.0 D[13]. The observed dipole moments of the amides, which are almost double those of the esters, are in the range 3.5–3.8 D[12]. The amide derivatives of ibuprofen would, therefore, form more stable dipole–dipole complexes than the corresponding esters and would

lead to retention on the column and resolution of the amide enantiomers. The observed capacity factors and resolution support this assumption.

CONCLUSIONS

The enantiomers of a number of pharmacologically important α-methylaryl anti-inflammatory agents can be readily resolved as 1-naphthalenemethylamides on the (*R*)-N-(3,5-dinitrobenzoyl)phenylglycine CSP described by Prikle *et al.*[1]. The mechanism of this separation appears to require the formation of a CSP–solute complex that is based on dipole stacking between the amide portions of the CSP and the solute. Chiral discrimination is dependent on steric factors resulting from conformational adjustments of the molecules within the CSP–solute complex. The proposed model, which is consistent with and supportive of the model proposed by Pirkle *et al.*[2,3], can be used to design appropriate derivatives for other chiral amines and acids and should expand the applications of this CSP.

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