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Chromatographic and ^{1}H NMR support for a proposed chiral recognition model

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Abstract

Liquid chromatography and ^{1}H NMR spectroscopy were used in an investigation of a chiral recognition rationale which was previously advanced to account for the resolution of naproxen (NAP-COOH) enantiomers on brush-type chiral stationary phases, **CSP-1** and **CSP-2**, identical except for tether length. **CSP-1** has recently been commercialized as the Whelk-O 1. This chiral stationary phase (CSP), basically an immobilized enantiomer of N-(3,5-dinitrobenzoyl)-4-amino-1,2,3,4-tetrahydrophenanthrene, was designed to have a cleft in which one enantiomer is preferentially bound. The cleft consists of π-acidic and π-basic aromatic systems held more or less perpendicular to each other. Aromatic substituents in the analyte were expected to be held in this cleft by simultaneous face-to-face and face-to-edge $\pi-\pi$ interactions. To ascertain whether analytes are truely bound in this cleft, NMR studies of mixtures of several naproxen-like analytes and chiral solvating agent **3**, a soluble version of the selector used in **CSP-1**, were undertaken. Additional motivation for the study came from the observation that the enantiomers of NAP-COOH and NAP-COOMe elute in a different order than do the enantiomers of NAP-CONHMe, and NAP-CON(Me)$_2$. This difference in the elution order of the amide derivatives with respect to the esters and free acid was not totally unexpected, for the chiral recognition hypothesis used in the design of the chiral selector allowed for such an eventuality. It was known from reciprocal chromatographic studies that the enantiomers of soluble analogs of the Whelk-O 1 CSP show different elution orders on naproxen-derived amide CSPs than they do on naproxen-derived ester CSPs. These data aided in formulation of the initial chiral recognition rationale. Evidence for the occurance of the specific molecular interactions suggested by this rational is provided by the presently described ^{1}H NMR study of the enantioselective complexation of the enantiomers of NAP-COOH, NAP-COOMe, NAP-CONHMe, and NAP-CON(Me)$_2$ by a single enantiomer of a soluble analog of the Whelk-O 1 CSP.

1. Introduction

Chiral stationary phases (CSPs) **1** and **2**, designed for the chromatographic separation of the underivatized enantiomers of the commercially important 2-arylpropionic acid, naproxen [1,2], afford excellent separations not only for the underivatized enantiomers of naproxen but also for a number of related non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, fenoprofen and flurbiprofen. Moreover, CSP **2** is useful for separating the enantiomers of

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a large number of compounds of diverse types [3]. The mechanistic concepts underlying the design of CSPs **1** and **2** were developed largely through the use of chromatographic data. The final design was reached with the aid of CPK[1] space-filling models. The essential aspect of the design is to incorporate a cleft in which face-to-face and face-to-edge $\pi-\pi$ interactions occur simultaneously with an aromatic substituent present near the stereogenic center of the analyte. A proximate hydrogen bond acceptor site is also required by the chiral recognition rationale. This paper reports the results of an ^{1}H NMR solution study of the structure of the complexes derived from naproxen (NAP-COOH) and naproxen derivatives (NAP-COOMe, NAP-CONHMe, NAP-CON(Me)$_2$) and the chiral solvating agent (CSA) **3**, a soluble analogue of the selector used in CSPs **1** and **2** (Fig. 1).

An illustration of the rationale advanced to account for the observed separation of naproxen enantiomers on CSPs **1** and **2** is presented in Fig. 2 [1,2]. Four possible diastereomeric adsorbates derived from an (*S*,*S*)-CSP and the two enantiomers of naproxen are represented using computer-generated space-filling molecular model representations. The alkyl tether of the CSP is represented as an *n*-propyl substituent, and distances between the two components of the diastereomeric complexes have been exaggerated for the sake of clarity. Each of the component molecules is represented in a conformation which is presumed to be of relatively low energy and hence extensively populated. The dihedral angle between the methine hydrogen and the carbonyl oxygen of naproxen and its derivatives is taken to be *ca.* 180°. The dinitrobenzoyl ring system of the (*S*,*S*)-CSP is viewed edge on, with the amide hydrogen projecting toward the viewer.

Each of the four adsorbate pairs pictured in Fig. 2 are held together by a combination of hydrogen bonding and $\pi-\pi$ interaction forces. The hydrogen bond formed between the amide hydrogen of the CSP and the carboxylate oxygen of the analyte molecule can occur at either of the two oxygens of the carboxylate system. These adsorbates are designated as **C** and **H**, depending on whether the hydrogen bond occurs at the carbonyl oxygen or the hydroxyl oxygen. Furthermore, the face to face $\pi-\pi$ interaction can involve interaction of the naphthyl ring of naproxen (or, in the general case, the aryl substituent of the NSAID) with either face of the dinitrobenzamide system of the CSP, resulting in

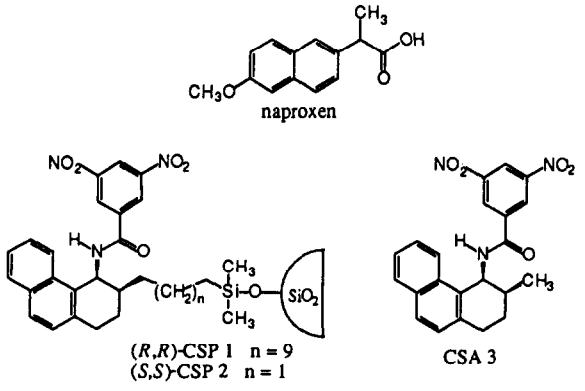


Fig. 1. Structures of CSPs **1** and **2** and CSA **3** used in the study.

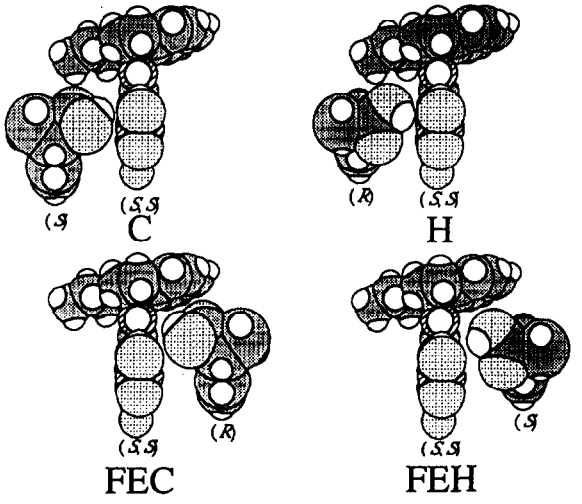


Fig. 2. Computer-generated space-filling molecular model representations of the four diastereomeric adsorbates proposed to account for separation of naproxen enantiomers on CSPs **1** and **2**.

[1] Corey–Pauling–Koltun, CPK® Models are available from Harvard Apparatus, Inc., 22 Pleasant Street, South Natick, MA 01760, USA.

the four possible adsorbates pictured in Fig. 2. CSPs **1** and **2** have been designed so as to encourage binding within the "cleft" formed by the two aromatic systems. Binding within this cleft is believed to allow the formation of a face-to-edge $\pi-\pi$ interaction (**FE**) involving the dihydrophenanthrene ring of the CSP and the aryl ring of the NSAID. The shortened tether of CSP **2** may provide further preference for the binding of analytes within this cleft, since the formation of adsorbates **C** and **H** may be sterically impeded by the underlying silica support [2].

Adsorbate **FEH** is believed to illustrate the predominant mode of interaction between the CSP and the more retained enantiomer of naproxen. By utilizing the hydroxyl oxygen as a hydrogen bonding site, the methine hydrogen is directed toward the dihydrophenanthrene portion of the CSP. The small size of this hydrogen permits the two components to approach closely where the slightly acidic methine hydrogen may undergo weak bonding to the π-cloud of the dihydrophenanthrene system. Additionally, the hydroxyl group may simultaneously hydrogen bond to the proximate nitro group. From the models, it would appear that the hydroxyl proton in adsorbate **FEH** is positioned so as to allow a trifurcated hydrogen bond between the two carboxyl and one of the nitro group oxygens. No experimental evidence is offered for this however. Adsorbate **FEC** is believed to illustrate the predominant adsorbate formed by the less retained enantiomer.

The naproxen derivatives illustrated in Fig. 3 were prepared in order to test the chiral recognition rationale presented in Fig. 2. Unlike NAP-COOH and NAP-COOMe, the amide derivatives NAP-CONHMe and NAP-CON(Me)$_2$ contain but one oxygen which can serve as a hydrogen bond accepting site, thus only adsorbates **C** and **FEC** need be considered. Considerations of both steric encumbrance to adsorbate formation and the potential contribution of energetically favorable face to edge $\pi-\pi$ interaction suggest that the heterochiral [(*S*,*S*)-CSA · (*R*)-analyte] adsorbate **FEC** should be more stable. Thus, for amide derivatives of naproxen, CSPs **1** and **2** are expected to selectively retain the enantiomer which affords the heterochiral adsorbate, whereas the enantiomer which affords the homochiral [(*S*,*S*)-CSA · (*S*)-analyte] adsorbate is known to be retained in the case of the free acid [1,2]. Ester derivatives, like the free acid, contain two oxygens, thus all four adsorbates pictured in Fig. 2 must be considered. However, since the oxygen involved in hydrogen bonding in adsorbate **FEH** is sterically less accessible in the ester than in the acid and the ester cannot utilize the postulated trifurcated hydrogen bond, one might anticipate somewhat attenuated enantioselectivity for the ester relative to the acid.

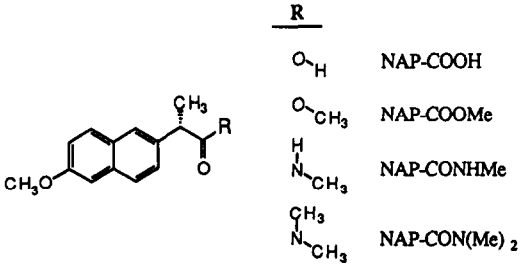


Fig. 3. Derivatives of (*S*)-naproxen used in the study. Small amounts of the corresponding compounds derived from racemic naproxen were also prepared for the HPLC study.

2. Experimental

2.1. General methods

All reagents were of pharmaceutical or reagent grade and were used without further purification. Solvents used were HPLC grade or distilled prior to use. (*S*)-Naproxen, (+)-6-methoxy-α-methyl-2-naphthyleneacetic acid, was obtained from Aldrich (Milwaukee, WI, USA). Racemic naproxen was kindly donated by Sepracor (Marlborough, MA, USA). The preparation of CSPs **1** [1] and **2** [2] has been described previously. NAP-COOMe was prepared via Fischer esterification with methanol. Amide derivatives NAP-CONHMe and NAP-CON(Me)$_2$ were prepared from the corresponding acid chloride, which was generated using oxalyl chloride. Chromatographic analysis was performed using an Altex Model 100A pump, a Rheodyne Model

7125 injector with a 20-μl sample loop, a Linear UVIS 200 variable wavelength absorbance monitor set at 254 nm, and a Hewlett-Packard HP 3394 integrating recorder. All chromatographic experiments were carried out at a nominal flow-rate of 2.00 ml/min. Column void time was measured by injection of 1,3,5-tri-*tert.*-butylbenzene. All ^{1}H NMR chemical shifts are reported in ppm (δ) relative to tetramethylsilane.

3. Results and discussion

3.1. HPLC studies

Data pertinent to the separation of the enantiomers of the naproxen derivatives on CSPs **1** and **2** are shown in Table 1. The ester derivative, NAP-COOMe, shows retention of the enantiomer forming the homochiral adsorbate [i.e. the (*R*) enantiomer is selectively retained on an (*R*,*R*)-CSP, and the (*S*) enantiomer is selectively retained on an (*S*,*S*)-CSP]. The enantioselectivity observed for the ester is somewhat smaller than that observed for the free acid. The naproxen amide derivatives show retention of the enantiomer forming the heterochiral adsorbate, with the dimethylamide, NAP-CON(Me)$_2$, showing greater enantioselectivity than the methylamide, NAP-CONHMe. These results lend support to the suggestion that adsorbate **FEH** may be the preferred mode of interaction of free acid and methyl ester with the CSP, whereas adsorbate **FEC** may be the preferred mode of interaction of the amide derivatives.

Further support the for this chiral recognition rationale is provided by studies of the reciprocal chromatographic situation, in which a variety of amide, ester and ionic-linked naproxen-derived CSPs were used to resolve the enantiomers of soluble analogues of the CSPs **1** and **2** [4].

3.2. ^{1}H NMR studies

Examination of molecular models of proposed adsorbates **FEC** and **FEH**, believed to account for the predominant adsorption of naproxen and its derivatives on CSPs **1** and **2**, suggests that ^{1}H NMR solution studies should afford some insight into the chiral recognition process. In adsorbate **FEC**, the methine hydrogen of naproxen is oriented away from the tetrahydrophenanthrene ring of the CSP whereas in adsorbate **FEH**, this hydrogen is forced into the π-cloud of the tetrahydrophenanthryl ring. One would expect that an upfield chemical shift would be observed for this methine signal in cases where adsorbate **FEH** is formed to a great extent. In cases where formation of adsorbate **FEC** is favored, the methyl group of naproxen is similarly expected to be shifted upfield.

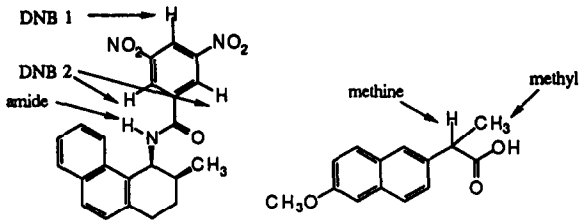
Solutions of a 1:1 mixture of either enantiomer of CSA **3** with each of the four (*S*)-naproxen analytes shown in Fig. 3 were prepared as 12.5 m*M* solutions in deuterochloroform and examined by ^{1}H NMR spectroscopy at ambient temperature. Table 2 shows the major chemical

Table 1
Separation of the enantiomers of naproxen derivatives on (*R*,*R*)-CSP **1** and (*S*,*S*)-CSP **2**

Compound	(*R*,*R*)-CSP **1**			(*S*,*S*)-CSP **2**		
	k'_1	α	Retained[a]	k'_1	α	Retained[a]
NAP-COOH	4.73	2.15	Homochiral	2.88	2.98	Homochiral
NAP-COOMe	2.31	1.35	Homochiral	3.42	1.42	Homochiral
NAP-CONHMe	13.48	1.45	Heterochiral	18.73	1.41	Heterochiral
NAP-CON(Me)$_2$	4.24	2.61	Heterochiral	5.24	3.24	Heterochiral

Mobile phase, 20% 2-propanol in hexane with 1 g/l ammonium acetate; flow-rate, 2.00 ml/min; ambient temperature. k'_1 = Retention factor for initially eluted enantiomer; α = separation factor.
[a] Absolute configuration of retained enantiomer relative to absolute configuration of the CSP.

Table 2
Changes in ^{1}H NMR chemical shifts upon interaction of the four (*S*)-naproxen derivatives with either enantiomer of CSA **3**



Complex	CSA **3**			Naproxen		
	DNB 1	DNB 2	Amide	Methine	Methyl	Stability[a]
(*S*)-NAP-COOH + (*R*,*R*)-CSA	−0.05	−0.02	+0.05	−0.02	−0.03	Less
(*S*)-NAP-COOH + (*S*,*S*)-CSA	−0.08	−0.05	+0.11	−0.17	−0.05	More
(*S*)-NAP-COOMe + (*R*,*R*)-CSA	−0.07	−0.04	+0.14	−0.03	−0.10	Less
(*S*)-NAP-COOMe + (*S*,*S*)-CSA	−0.10	−0.05	+0.22	−0.24	−0.10	More
(*S*)-NAP-CONHMe + (*R*,*R*)-CSA	−0.14	−0.04	+0.53	−0.10	−0.17	More
(*S*)-NAP-CONHMe + (*S*,*S*)-CSA	−0.16	−0.04	+0.62	−0.40	−0.14	Less
(*S*)-NAP-CON(Me)$_2$ + (*R*,*R*)-CSA	−0.20	−0.07	+0.64	−0.09	−0.19	More
(*S*)-NAP-CON(Me)$_2$ + (*S*,*S*)-CSA	−0.06	−0.02	+0.24	−0.02	−0.03	Less

Conditions: analyte concentrations, 12.5 m*M* in C^2HCl_3; spectrometer frequency, 200 MHz; temperature, ambient.
[a]Relative stability of the diastereomeric complexes as inferred from chromatographic studies.

shift differences induced in CSA **3** and the naproxen derivatives through their interactions. The induced chemical shifts of the proton in the 4-position and of the two protons in the 2- and 6-positions of the dinitrobenzoyl ring of CSA **3**, reported as DNB 1 and DNB 2 in Table 2, are indicative of $\pi-\pi$ interaction, for mutual upfield chemical shifts are often noted upon formation of face to face $\pi-\pi$ complexes. The downfield shift of the amide hydrogen of CSA **3** reflects its participation in hydrogen bonding. Changes in the chemical shift of the methine and α-methyl protons of the various naproxen derivatives are expected to be sensitive to shielding effects from the tetrahydrophenanthrene ring of CSA **3** as described above.

The enantiomer of NAP-COOH which gives rise to the homochiral adsorbate is known to be preferentially retained on both CSPs **1** and **2**. Similarly, it is the homochiral complex of (*S*)-NAP-COOH and (*S*,*S*)-CSA **3** which shows a larger downfield shift in the amide hydrogen resonance and larger upfield shifts in the DNB proton resonances, both indicative of more extensive complexation. The homochiral complex also shows a larger upfield shift in the naproxen methine resonance, presumably owing to the previously discussed shielding to be expected in adsorbate **FEH**. NAP-COOMe also shows larger shifts in the amide, DNB and methine resonances for the more stable homochiral diastereomeric pair, again supporting the original chiral recognition rationale.

As in the chromatographic studies, it is the heterochiral adsorbate pair of NAP-CON(Me)$_2$ which is the most stable, as evidenced by the large shifts in the amide and DNB resonances observed for the heterochiral complex. In the previously discussed chiral recognition rationale, adsorbate **FEC** is presumed to be the predominant retention mode for amide derivatives of naproxen. This model predicts that shielding of the methine proton, which was important in the acid and ester derivatives, should be relatively unimportant for the amides, whereas some shielding of the naproxen methyl group at the stereogenic center may be expected. The results show that the methine hydrogen is indeed rela-

tively unaffected by complexation and that the methyl group is shifted upfield by nearly 0.2 ppm in the heterochiral complex.

All of the chemical shift perturbations observed in the ^{1}H NMR spectra of the diastereomeric complexes of NAP-COOH, NAP-COOMe, and NAP-CON(Me)$_2$ with CSA **3** support the chiral recognition rationale originally based upon chromatographic data and examination of molecular models. However, the behavior of NAP-CONHMe is somewhat surprising. Large shifts in DNB and amide hydrogen resonances are observed in both diastereomeric complexes. Furthermore, the less stable homochiral adsorbate pair (based upon chromatographic analysis) shows an extremely large upfield shift for the methine resonance, a result which cannot easily be reconciled with the proposed model. These results suggest that both of the diastereomeric complexes are quite stable. While the predominant heterochiral adsorbate is believed to be as pictured in adsorbate **FEC**, the nature of the homochiral adsorbate is unknown. Examination of molecular models suggests that an adsorbate structure similar to adsorbate **FEH** in which the amide hydrogen of NAP-CONHMe is directed into the π-cloud of the tetrahydrophenanthrene ring of CSA **3** may account for the enhanced stability of the homochiral complex. Such hydrogen bonding to the π-clouds of aromatic systems has been proposed to be important in other chiral recognition systems [5] and has received a good deal of recent attention [6,7].

Chromatographic analysis of naproxen derivatives was carried out on CSPs **1** and **2** using chloroform as eluent. The results, summarized in Table 3, shed some light upon the interesting behavior of NAP-CONHMe in the NMR study.

The results presented in Table 3, when contrasted with the results presented in Table 2, demonstrate the influence of mobile phase composition on the chromatographic separation of the enantiomers of naproxen derivatives. Interestingly, the enantiomers of NAP-CONHMe, although relatively strongly retained, are not resolved on either CSP **1** or CSP **2** when chloroform is used as a mobile phase. This result is consistent with the ^{1}H NMR data which indicates that the diastereomeric adsorbates derived from either enantiomer of CSA **3** and (*S*)-NAP-CONHMe are both quite stable, differing negligibly in this regard in chloroform.

4. Conclusions

A variety of chromatographic evidence, as well as ^{1}H NMR studies using a chiral solvating agent, has been used to investigate the mechanism of enantioselective retention of naproxen and its derivatives by the recently developed CSPs **1** and **2**. These investigations support the original chiral recognition rationale advanced to account for these separations. The current study provides insight which may prove useful both for understanding the resolution of non-NSAID analytes on CSP **2** (CSP **2** is commercially available as the Whelk-O 1 CSP from Regis Technologies) and for the design of improved CSPs.

Table 3
Chromatographic separation of the enantiomers of naproxen derivatives using chloroform as a mobile phase

Compound	CSP β1			CSP **2**		
	k_1'	α	Retained[a]	k_1'	α	Retained[a]
NAP-COOMe	0.19	1.37	Homochiral	0.28	1.43	Homochiral
NAP-COONHMe	1.80	1.00	–	1.75	1.00	–
NAP-CON(Me)$_2$	0.64	1.66	Heterochiral	0.51	2.24	Heterochiral

Conditions: mobile phase, 100% chloroform; flow-rate, 2.00 ml/min; temperature, ambient. k_1' = Retention factor for initially eluted enantiomer; α = separation factor.

[a] Absolute configuration of retained enantiomer relative to absolute configuration of CSP.

Acknowledgements

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