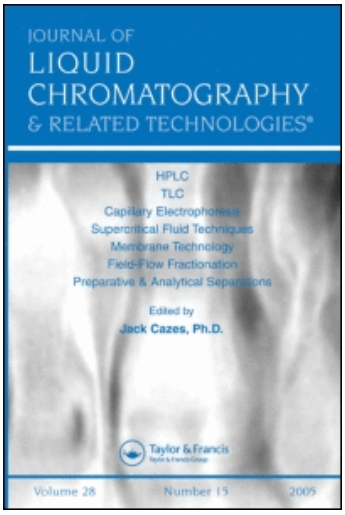

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Shimada, Kazutake , Haniuda, Emi , Oe, Tomoyuki and Nambara, Toshio(1987) 'Ferrocene Derivatization Reagents for Optical Resolution of Carboxylic Acids by High-performance Liquid Chromatography with Electrochemical Detection', Journal of Liquid Chromatography & Related Technologies, 10: 14, 3161 — 3172
To link to this Article: DOI: 10.1080/01483918708068304
URL: http://dx.doi.org/10.1080/01483918708068304

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(14), 3161-3172 (1987)

FERROCENE DERIVATIZATION REAGENTS FOR OPTICAL RESOLUTION OF CARBOXYLIC ACIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

New derivatization methods using chiral ferrocene reagents have been developed for the optical resolution of carboxylic acids by high-performance liquid chromatography with electrochemical detection. Two chiral derivatization reagents, 1-ferrocenylethylamine and 1-ferrocenylpropylamine, were readily prepared from acetylferrocene and propionylferrocene in two steps, respectively. Condensation of carboxylic acids with the chiral reagent was effected in the presence of water-soluble carbodiimide and 1-hydroxybenzotriazole. The diastereomeric amides formed from N-acetylamino acid and α-arylpropionic acid enantiomers were efficiently resolved by reversed-phase chromatography and showed the satisfactory sensitivity at +0.45 V vs. an Ag/AgCl reference electrode with a detection limit of 0.5 pmole (S/N=5).

INTRODUCTION

High-performance liquid chromatography (HPLC) with electrochemical detection (ECD) is a useful tool for the trace analysis

of various compounds in biological fluids. In recent years, pre- and post-column labeling methods have been developed to extend its applicability [1-7]. In the previous papers of this series we proposed novel ferrocene reagents for pre-column labeling of amino [2], hydroxyl [4], carboxylic [5, 6], and thiol [7] groups in HPLC/ECD. As the ferrocene derivative undergoes facile oxidation and the product is in turn readily reduced, it can be detected selectively in the presence of electroactive compounds such as phenols, catechols, and aromatic amines.

The present paper deals with the development of derivatization methods using R-(-)-1-ferrocenylethylamine and S-(+)-1-ferrocenylpropylamine which possess the ferrocene moiety as an electrophore and the applicability of these reagents to the resolution of N-acetylamino acid and α-arylpropionic acid enantiomers by reversed-phase HPLC/ECD.

MATERIALS AND METHODS

Materials

N-Acetylamino acids were purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan). Optically active ibuprofen was prepared by fractional crystallization of (+)- or (-)-1-methylbenzylamine salt of the commercially available racemate. Naproxen racemate and enantiomers were obtained by the known method as reported previously [8, 9]. (-)-2, 3: 4, 6-Di-O-isopropylidene-2-keto-L-gulonic acid hydrate ((-)-DAG) was supplied by Aldrich Chem. Co. (Milwaukee, WI, U.S.A.). Other reagents were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Solvents were purified by distillation prior to use.

Instruments

All melting points were taken on a micro hot-stage apparatus and are uncorrected. The optical rotations were measured

with a JASCO DIP-4 automatic polarimeter (JASCO, Tokyo). Mass (MS) spectral measurements were run on a Hitachi M-52 spectrometer (Hitachi Ltd., Tokyo). HPLC was carried out on a Waters Model 510 solvent delivery system (Waters Assoc., Milford, MA, U.S.A.) equipped with a Yanagimoto VMD-501 electrochemical detector having twin electrode in series system (Yanagimoto Co., Kyoto). The applied potential of the detector was set vs. an Ag/AgCl reference electrode. A Develosil ODS-5 (5 μm) column (15 cm x 0.4 cm i.d.) (Nomura Chemical Co., Seto, Japan) was used at ambient temperature. The pH of mobile phase was adjusted with AcOH. Dead time (t_0) was estimated by the use of $NaNO_2$.

Syntheses of Derivatization Reagents

1-Ferrocenylethylamine (Ia) and 1-ferrocenylpropylamine (IIa)----- 1-Ferrocenylethylamine (Ia) and 1-ferrocenylpropylamine (IIa) were prepared from acetylferrocene and propionylferrocene [10], respectively, according to the procedure described by Tverdokhlebov et al. [11]. Hydrogen chloride gas was passed into a solution of Ia and IIa in ether to give crystalline products. Recrystallization from MeOH gave Ia·HCl and IIa·HCl as yellow needles. Ia·HCl: mp 163-165°C (decomp.). Anal. Calcd for $C_{12}H_{16}ClFeN$: C, 54.27; H, 6.07; N, 5.27. Found: C, 54.20; H, 5.98; N: 5.40. IIa·HCl: mp 152-156°C (decomp.). Anal. Calcd for $C_{13}H_{18}ClFeN$: C, 55.85; H, 6.49; N, 5.01. Found: C, 55.71; H, 6.53; N, 5.10.

Optical resolution of Ia and IIa----- Optical resolution of Ia and IIa was done with (-)-DAG according to the procedure described by Herrmann et al. [12]. (-)-DAG salt of Ia: mp 224-226°C (from MeOH: Lit. mp 223°C [12]). (-)-DAG salt of IIa: mp 169-175°C (from MeOH: Lit. mp 175°C [12]). These salts were decomposed with 10% NaOH and the yielded bases were extracted with ether. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated down. R-(-)-1-Ferrocenylethylamine

(Ib): $[\alpha]_D^{21}$ -25.5° (c=2.12 in benzene) (Lit. $[\alpha]_D^{20}$ -26.3° (c=1 in benzene) [12]). S-(+)-1-Ferrocenylpropylamine (IIb): $[\alpha]_D^{29}$ +59.2° (c=0.60 in benzene) (Lit. $[\alpha]_D^{20}$ +58.0° (c=1 in benzene [12]). The optical purity of each amine was over 99.0% as judged by HPLC.

Derivatization

N-Acetylamino acids----- To a solution of N-acetylamino acid (2.5 μg) in pyridine (0.05 ml) were added Ib or IIb (300 μg) in CH_2Cl_2 (0.1 ml), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (water-soluble carbodiimide; WSC, 1 mg) in CH_2Cl_2 (0.1 ml) and 1-hydroxybenzotriazole (HOBT, 50 μg) in CH_2Cl_2/pyridine (4:1, 0.1 ml), and the whole was allowed to stand at 4°C for 2 hr. An aliquot of the resulting solution was injected into the chromatograph.

Ibuprofen and naproxen----- A solution of ibuprofen or naproxen (2.5 μg) in tetrahydrofuran (0.05 ml) was treated in the same manner as described above. An aliquot of the resulting solution was injected into the chromatograph.

Preparation of Standard (±)-Ibuprofen Derivative with Ib

(±)-Ibuprofen (ca. 2 mg) in tetrahydrofuran (0.1 ml) was treated with Ib (ca. 20 mg) in CH_2Cl_2 (0.1 ml) and WSC (20 mg) in CH_2Cl_2 (1 ml) in the similar manner as described above. The reaction mixture was diluted with ether and washed successively with 5% HCl, 2% NaOH, and H_2O. Removal of the solvent with the aid of an N_2 gas stream gave the yellow solid (ca. 2 mg). MS m/z 417 (M^+).

RESULTS AND DISCUSSION

The design of a promising derivatization reagent for the liquid chromatographic resolution of carboxylic acid enantiomers

via diastereomers with ECD requires the incorporation of suitable structural features: chirality leading to efficient resolution, a reacting group toward the carboxylic acid function, and an electrophore responding to the ECD with the satisfactory sensitivity. In the present study, two chiral secondary amines, R-(-)-1-ferrocenylethylamine (Ib) and S-(+)-1-ferrocenylpropylamine (IIb) possessing ferrocene as an electrophore have been used for this purpose. 1-Ferrocenylethylamine (Ia) and 1-ferrocenylpropylamine (IIa) were prepared from acetylferrocene and propionylferrocene, respectively, and the optical resolution was attained by repeated fractional crystallization of the (-)-DAG salt (Fig. 1).

The applicability of these reagents to the separation of carboxylic acid enantiomers by HPLC was then investigated. HPLC/ECD has been usually limited to reversed-phase or ion-exchange chromatography because of the solubility of supporting electrolytes. Reversed-phase and ion-exchange chromatographic methods are less favorable than normal-phase chromatography for the separation of diastereomers [8]. In contrast, reversed-phase chromatography is applicable to the polar compounds and substances in polar solvents, whose separation is difficult in normal-phase chromatography. The use of organic solvents is also one of disadvantages in normal-phase chromatography. Recently several attempts to separate diastereomers on a highly efficient reversed-phase column have been reported [13]. Therefore, a reversed-phase column, Develosil ODS-5, was used in the present study. Condensation of N-acetylamino acid with the chiral reagent was effected in the presence of WSC and HOBT. The capacity factor (k') and resolution (R) values for four pairs of diastereomers produced with Ib and IIb are listed in Table 1. It is evident from the data that the complete separation was attained for all the pairs of N-acetylamino acids. The elution order of D- and L-N-acetylalanines was reversed from that of other N-acetylamino acids derivatized with Ib or IIb. The satisfactory separation

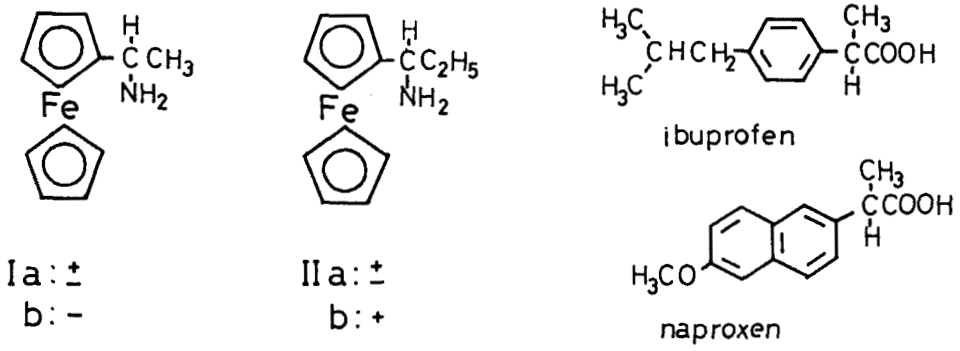

FIGURE 1. Structures of Derivatization Reagents and α-Aryl-propionic Acids.

TABLE 1
HPLC Separation of Diastereomeric Amides Derived from N-Acetylamino Acids with Ferrocenylalkylamines

N-Acetylamino acid		Ib		IIb	
		k'	R	k'	R
Alanine	D	13.2[a]		28.8[c]	
	L	12.0	1.20	32.0	1.50
Valine	D	5.4[b]		6.4[d]	
	L	6.4	1.33	5.2	1.70
Leucine	D	9.6[b]		5.8[d]	
	L	12.4	2.40	4.6	1.50
Phenylalanine	D	12.4[b]		7.4[d]	
	L	16.4	2.67	5.6	1.80

Conditions: mobile phase, tetrahydrofuran/1.5% AcONa (pH 5.0) a) (1:4), b) (1:2), c) (1:4), d) (2:3). Flow rate was set at 0.5 ml/min (t_0=2.5 min) except for c) (1 ml/min, t_0=1.25 min).

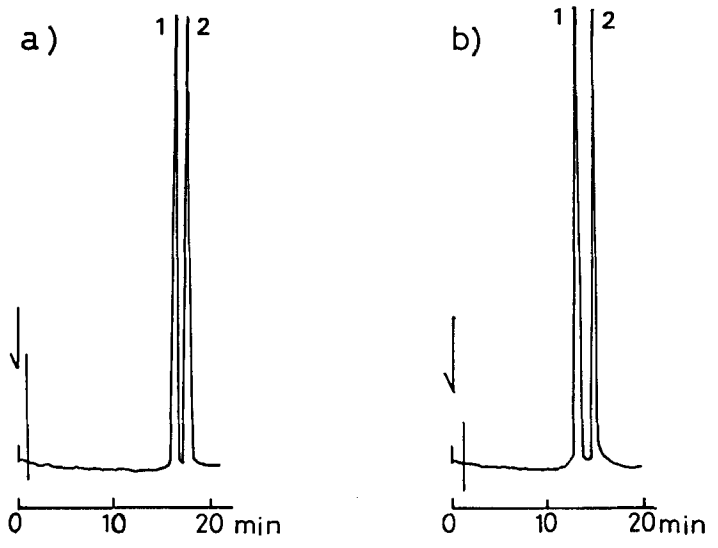

FIGURE 2. High-Performance Liquid Chromatograms of Diastereomeric Amides Formed with R-(-)-1-Ferrocenylethylamine. a) 1: (-)-Ibuprofen, 2: (+)-ibuprofen; b) 1: (-)-naproxen, 2: (+)-naproxen.
Conditions: mobile phase, acetonitrile/1.5% AcONa (pH 5.0)
a) (3:2), b) (5:4), 1.5 ml/min (t_0=0.8 min).

was obtained with IIb in somewhat shorter retention time than that with Ib except for N-acetylalanine.

This derivatization method was further applied to the separation of α-arylpropionic acid, (±)-ibuprofen, which is currently used as an anti-inflammatory drug. The diastereomers formed with Ib were completely separated on a Develosil ODS-5 column with acetonitrile/1.5% AcONa (pH 5.0) (3:2) as a mobile phase (R= 1.75), exhibiting good sensitivity with a detection limit of 0.5 pmole (S/N=5 at 4 nA full scale) (Fig. 2a). On the contrary, the diastereomer with IIb showed poor resolution under several conditions examined. Another α-arylpropionic acid, (±)-naproxen, was

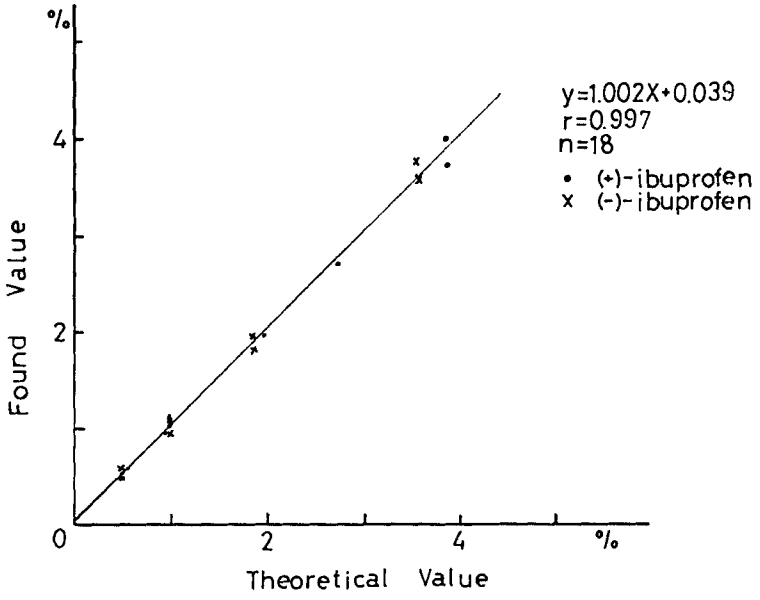

FIGURE 3. Recovery Tests for (+)- or (-)-Ibuprofen Added to Its Enantiomer (2.5 μg).
The composition ratio of each enantiomer added was expressed in percentage.

also distinctly resolved with Ib (R=1.75) as shown in Figure 2b. The diastereomeric amides formed from (-)-enantiomers of these drugs were eluted earlier. Condensation of (±)-ibuprofen with Ib was readily attained in the presence of WSC and HOBT at 4°C. The resulting solution could be directly applied to HPLC without exerting any disturbance on the chromatogram. The amount of diastereomer was estimated by comparison with the peak area of the synthetic standard sample. The yield of the diastereomeric amides was raised with increasing reaction time and reached a plateau in 120 min. No racemization of the product or derivatization reagent occurred, even under prolonged reaction condi-

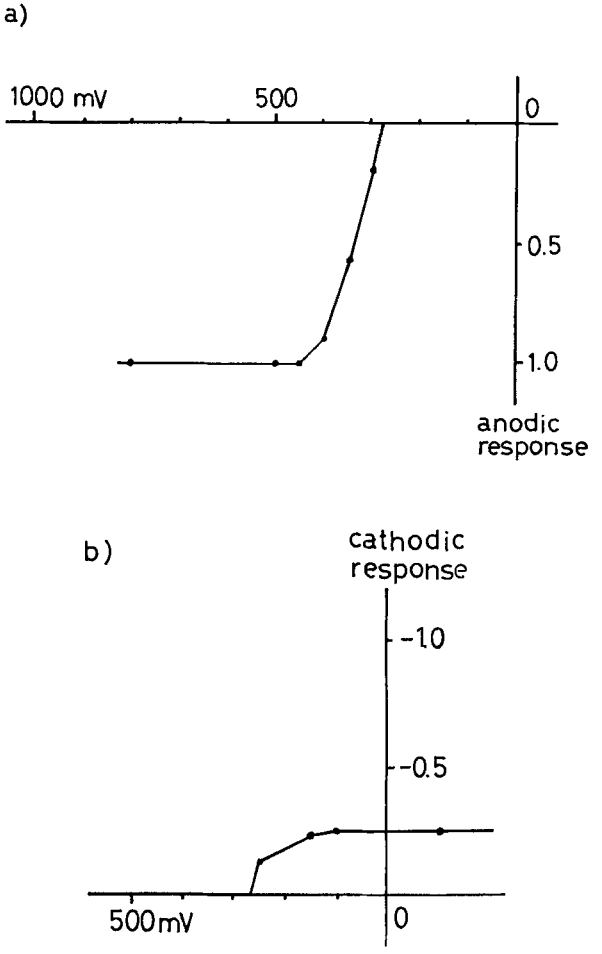

FIGURE 4. Hydrodynamic Voltammograms of (±)-Ibuprofen Derivatives.
a) Anodic response, b) cathodic response: upstream electrode +0.6 V.

tions. When varying amounts of (+)- or (-)-ibuprofen were added to a certain amount of its enantiomer and determined, a good correlation was observed between theoretical and found values of the former (Fig. 3). As little as 0.5% of one enantiomer can be detected in the presence of 99.5% of the other. The electrochemical properties of the amide with Ib were investigated using (±)-ibuprofen as a model compound. The half-wave potentials ($E_{1/2}$) of both diastereomeric amides were +0.33 V and the oxidation products from the amides were reduced at the downstream electrode (Fig. 4). These data imply that the amides can be detected selectively in the presence of common electroactive compounds, such as phenols, catechols, and aromatic amines [6].

In conclusion, these chiral derivatization reagents proved to be satisfactory for the resolution of carboxylic acid enantiomers and their sensitive electrochemical monitoring in reversed-phase HPLC. Although a variety of derivatization methods have been developed for the resolution of enantiomers [8], this is the first reported instance using HPLC/ECD. Further application of the present method to the metabolic study of (±)-ibuprofen is being conducted in these laboratories and the details will be reported elsewhere.

ACKNOWLEDGEMENTS

The authors are indebted to Miss C. Sakayori for her technical assistance. We also express our thanks to the staff of the central analytical laboratory of this Institute for elemental analyses and spectral measurements. This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

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