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Enantiomeric separation of substituted 2-aryloxy propionic esters

Application to the determination of the enantiomeric excess in herbicide formulations

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ABSTRACT

The enantiomeric separation of twenty substituted 2-aryloxypropionic acid methyl esters was investigated using a π-acid chiral stationary phase derived from (*R,R*)-(N,N′-3,5-dinitrobenzoyl)-*trans*-1,2-diaminocyclohexane. Resolution factors in the range 2–4 were usually obtained. The parameters affecting the enantioselectivity (electronegativity and position of the aromatic substituents, nature and composition of the mobile phase) are discussed. Finally, the method was applied to the determination of the enantiomeric excess of an optically active herbicide formulation containing the *n*-butoxyethyl ester of 2-(4-chloro-2-methyl phenoxy)propionic acid.

INTRODUCTION

Substituted 2-aryloxypropionic acids (APAs) are the precursors of numerous herbicides produced in hundreds of thousands of tons annually [1,2]. Among them, methyl and 2-butoxyethyl (BOE) esters of 2-(4-chloro-2-methylphenoxy)propanoic acid (CMPP) and 2-(2,4-dichlorophenoxy)propanoic acid (2,4-DP) are the most important (Fig. 1).

The structure of these compounds contains an asymmetric centre, leading to two optical isomers. In the last few years, the different biological activities of each enantiomer have been evaluated, and it was found that the herbicidal activity is almost exclusively concentrated in the *R* form, the *S* form being herbicidally inactive [3].

Environmental considerations have recently led to the production and marketing of the single active *R* enantiomer. There is no doubt that this tendency will increase

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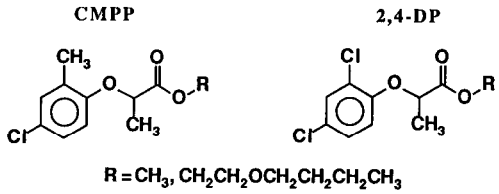


Fig. 1. Structures of CMPP and 2,4-DP esters.

in the next few years. Consequently, accurate analytical methods will be required in order to monitor the enantiomeric composition of herbicide formulations.

So far, the enantiomeric separation of numerous APA esters [methyl, ethyl, BOE, 2-ethylhexyl (EH)] has been investigated using three different chiral stationary phases (CSPs).

The resolution of a series of twenty APA methyl esters was carried out by Dernoncour and Azerad [4] using a covalently bound (*R*)-N-(3,5-dinitrobenzoyl)-phenylglycine (DNBPG) CSP. Selectivity values up to 1.26 were obtained, leading for some solutes (naphthyl derivatives) to baseline resolutions suitable for the determination of the enantiomeric excess. Similar results were also obtained by Müller and Bosshardt [3] with herbicides as methyl or BOE esters. A major contribution was made by Blessington and Crabb [1,2], who proposed a method for determining CMPP BOE and EH esters using an ionic DNBPG CSP.

Nevertheless, Müller and Bosshardt [3] claimed better results when using a Nucleosil Chiral-2 column [chiral selector based on tartaric acid and (dinitrobenzyl)-phenylethylamine]. Resolution factors in the range 2–4 were achieved for six herbicide APA esters.

Another approach was investigated by Gaffney *et al.* [5] using a Chiralcel OB CSP (cellulose tribenzoate coated on silica gel). The enantiomeric separation of 2-phenoxypropanoic acid methyl ester was carried out and was found to be closely related to the nature of the alcoholic polar modifier.

This work had a two-fold purpose: first, the ability of a CSP derived from (*R,R*)-(N,N′-3,5-dinitrobenzoyl)-*trans*-1,2-diaminocyclohexane (DACH-DNB) (Fig. 2), designed by Misiti and co-workers [6–8], to resolve a large series of 2-APA esters, and second, the determination of the enantiomeric composition of a CMPP BOE ester formulation.

EXPERIMENTAL

Apparatus

Liquid chromatography was performed using a modular liquid chromatograph (Gilson, Villiers-le-Bel, France) equipped with a Shimadzu C-R3A integrator (Touzart et Matignon, Vitry-sur-Seine, France). The standard operating conditions were flow-rate 2 ml/min UV detection at 230 nm (ethanol) or 254 nm (methylene chloride) and room temperature.

Chiral stationary phase

The structure of the DACH-DNB CSP is shown in Fig. 2. This CSP is obtained

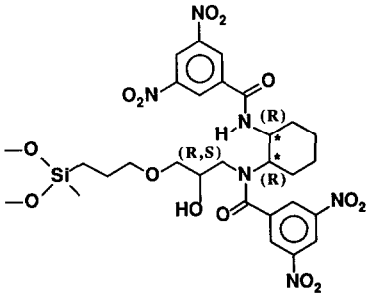


Fig. 2. Structure of the DACH-DNB CSP.

starting from 5-μm LiChrosorb Si-100 silica gel (Merck, Darmstadt, F.R.G.) modified with 3-glycidyloxypropyltrimethoxysilane [6–8]. For the present work, it was kindly provided by Professors D. Misiti and F. Gasparrini (University of Rome "La Sapienza") and packed into a 150 × 4.6 mm I.D. stainless-steel column by the classical slurry technique under 400 bar using ethanol as pumping solvent.

Mobile phase

Ethanol and hexane were of LiChrosolv grade (Merck) and methylene chloride [stabilized with 0.1% (w/w) of ethanol] of analytical-reagent grade was purchased from Prolabo (Paris, France).

Solutes

The methyl esters of APAs were kindly given by Dr. R. Azerad (Université René Descartes, Paris V, Paris, France) and the (±)- and (+)-BOE esters of CMPP and 2,4-DP by Mr. J. M. Pertuisot (Compagnie Française des Produits Industriels, Gennevilliers, France).

RESULTS AND DISCUSSION

Resolution of APA methyl esters

Chromatographic data for methyl esters are given in Table I. For each polar modifier, its content in the mobile phase (%), the capacity factor for the most retained enantiomer (k'_2), the selectivity value (α) and the resolution factor (R_s) are reported.

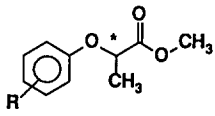
In all instances baseline resolution was achieved, except for nitro and carboxymethyl derivatives (solutes 16–18 and 14, respectively). The strong electron-withdrawing power of these substituents prevents the solutes from interacting with the CSP through a charge-transfer complex. It can therefore be assumed that the chiral recognition process of resolved compounds is partly governed by a π–π interaction, leading to high selctivity values for strong π-basic naphthyl derivatives.

This was evidenced for nine resolved solutes. The solutes listed in Table II differ only in the nature and/or the position of their aromatic ring substituent whose electronegativity can be quantified by a Hammet σ constant [9] (Table II). This constant was initially based on the ionization of substituted benzoic acids in water. The σ values defined by this reaction for *para* and *meta* substituents are constant characteristics of each substituent, independent of the nature of the reaction involved.

TABLE I

RESOLUTION OF APA METHYL ESTERS USING ETHANOL OR METHYLENE CHLORIDE AS POLAR MODIFIER

Column, 150 × 4.6 mm I.D.; mobile phase, *n*-hexane–ethanol or *n*-hexane–methylene chloride with ethanol and methylene chloride contents as indicated; flow-rate, 2 ml/min; UV detection at 230 nm with ethanol and 254 nm with methylene chloride; room temperature. Capacity factors k'_1 and k'_2 (for the first and the second eluted enantiomers, respectively) were calculated from the dead retention time t_0 (t_0 = 0.9 min, measured with heptane) using the equation $k' = (t_r - t_0)/t_0$. Selectivity: $\alpha = k'_2/k'_1$. Resolution: $R_s = 2(t_{r_2} - t_{r_1})/(\omega_1 + \omega_2)$ where ω is the baseline width.



No.	R	Ethanol content (%)	k'_2	α	R_s	CH_2Cl_2 content (%)	k'_2	α	R_s
1	H	0.125	7.05	1.41	2.8	5	9.08	1.43	2.8
2	3-CH_3	0.125	9.13	1.53	4.5	5	11.69	1.55	3.8
3	4-CH_3	0.125	9.74	1.57	4.7	5	9.90	1.65	5.2
4	2-Cl	0.125	7.54	1.26	2.3	5	8.24	1.51	2.7
5	3-Cl	0.125	5.81	1.29	2.0	5	8.05	1.35	2.2
6	4-Cl	0.125	6.55	1.40	2.4	5	9.18	1.46	2.7
7	2,4-Cl	0.125	5.33	1.23	2.2	5	7.17	1.30	2.1
8	2-CH_3, 4-Cl	0.05	7.35[a]	1.32	2.6	3	5.32[a]	1.29	1.7
9	2,4,5-Cl	0.125	4.26	1.18	1.6	5	5.33	1.24	1.5
10	2-OCH_3	1.25	6.55	1.18	1.8	15	7.47	1.36	2.6
11	3-OCH_3	1.25	5.05	1.20	2.0	15	5.55	1.50	2.8
12	4-OCH_3	1.25	5.61	1.24	2.2	15	6.67	1.48	2.6
13	2-$COOCH_3$	1.25	11.60	1.11	1.3	15	14.53	1.19	1.3
14	3-$COOCH_3$	1.25	7.66	1.10	0.92[b]	15	8.74	1.20	1.5
15	4-$COOCH_3$	1.25	9.26	1.13	1.3	15	11.02	1.29	2.1
16	2-NO_2	1.25	10.70	1.04	0.36[b]	15	15.75	1.13	1.4
17	3-NO_2	1.25	5.77	1	0	15	7.50	1	0
18	4-NO_2	1.25	9.08	1	0	15	14.29	1.07	0.67[b]
19	1-Naphthyl	1.25	6.73	1.31	2.6	15	6.55	1.75	4.8
20	2-Naphthyl	1.25	10.03	1.81	6.8	15	11.00	2.71	6.3

[a] *R*(+) enantiomer eluted first.

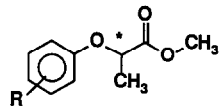
[b] For a pair of partially resolved peaks the value was calculated as (mean of peak heights − trough to baseline height)/mean of peak heights.

For any process, σ provides a measure of the total electronic influence of the substituent (polarity, electronegativity) [9,10]. The more electron-withdrawing the substituent, the higher is the value assigned to it. Accordingly, the σ value is a convenient means for determining the π-basicity or the π-acidity of a given aromatic ring and hence the magnitude of a charge transfer complex.

In Fig. 3, the logarithms of the selectivity values of compounds 1, 2, 3, 5, 6, 11, 12, 14 and 15 are plotted *versus* the Hammet σ values of their substituents. A similar experiment was carried out for the enantiomeric separation of N-arylsulphinamoyl acetates on a CSP derived from tyrosine [11]. A good correlation between log α and σ was obtained when using hexane–ethanol (99:1, v/v) as the mobile phase. The π–π interaction may thus be considered as the driving force of the chiral recognition

TABLE II

HAMMET σ VALUES ACCORDING TO REF. 9



Solute	R	σ
1	H	0
2	3-CH_3	−0.069
3	4-CH_3	−0.17
11	3-OCH_3	0.115
12	4-OCH_3	−0.268
5	3-Cl	0.373
6	4-Cl	0.227
14	3-$COOCH_3$	0.32
15	4-$COOCH_3$	0.39

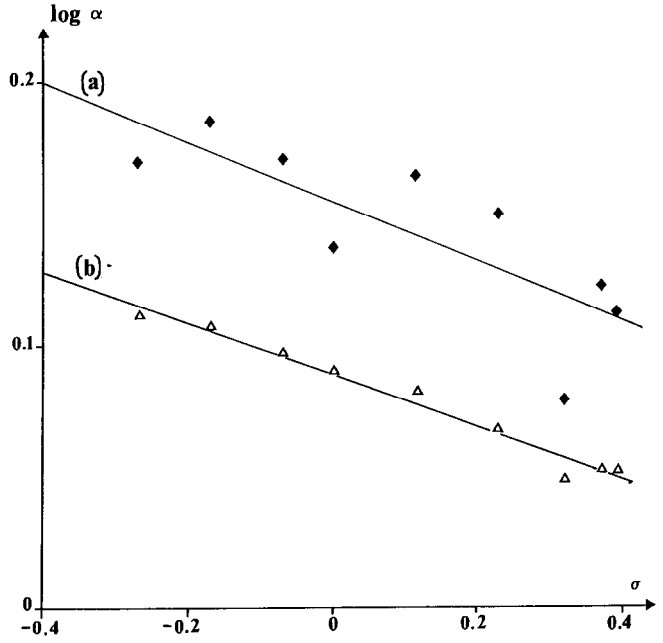


Fig. 3. Variation of log α with Hammet σ values (Table II) for compounds 1, 2, 3, 5, 6, 11, 12, 14 and 15 using (a) methylene chloride (15% in *n*-hexane) or (b) ethanol (1% in *n*-hexane) as polar modifier. Flow-rate, 2 ml/min; room temperature; UV detection at (a) 254 nm and (b) 230 nm.

mechanism. On the other hand, when using hexane–methylene chloride (85:15, v/v) as the mobile phase, this interaction seems to be of less importance (a poor correlation is obtained). The interactions between amide dipoles (hydrogen bonding or dipole stacking) prevail over the π–π interaction, probably because of their weaker solvation by methylene chloride than by ethanol. A similar conclusion was previously reached for N-arylsulphinamoyl acetates [11].

According to Table I, the retention of *ortho*-proton acceptor-substituted compounds (OCH_3, $COOCH_3$) is higher than that for the corresponding *para*- and *meta*-substituted compounds (solutes 10–15). Assuming that the polarities and the electronegativities of *ortho*- and *para*-substituted compounds are similar, it can be inferred that an additional hydrogen bonding takes place between the *ortho* substituent and a proton-donor site of the CSP. The only proton-donor site in the vicinity of the 3,5-DNB moiety of the CSP is the amidic proton. This is assumed to be involved in hydrogen bonding with the ethereal oxygen atom of the solute. This interaction is highly enantioselective as it hinders the free rotation around the C–O bond. The presence of a proton-acceptor substituent in the *ortho* position may compete with the formation of this interaction, leading to smaller selectivity values (Table I), especially with methylene chloride.

Selectivity was found to be dependent on the content of polar modifier in the mobile phase. In Fig. 4, the selectivity values obtained for solute 2 (R = 3-CH_3) are

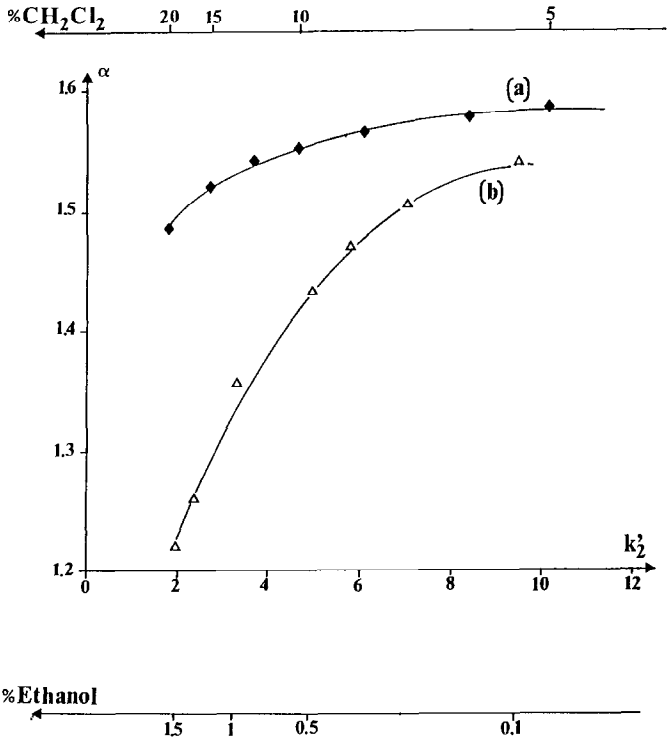


Fig. 4. Variation of α with k'_2 for solute 2 (R = 3-CH_3) using (a) methylene chloride (range 5–20%) or (b) ethanol (range 0.1–1.5%) as polar modifier in *n*-hexane. Other operating conditions as in Fig. 3.

plotted *versus* the capacity factor of the most retained enantiomer in the range 1–10. As reported previously for π-basic Pirkle-type CSPs [11,12], methylene chloride affords a greater enantioselectivity than ethanol. According to Pescher *et al.* [12], this phenomenon can be correlated with the fact that the amide dipoles of the CSP are more easily solvated by alcohols than by chlorinated solvents. Therefore, CSP–solute interactions are maximized when using the latter. According to Fig. 4, the enantioselectivity is much more affected by the ethanol content [$\Delta\alpha/\Delta k'_2 = 3.9\%$ and $\Delta\alpha/\Delta$(ethanol content) = 22.3%] than by methylene chloride [$\Delta\alpha/\Delta k'_2 = 1.2\%$ and $\Delta\alpha/\Delta$(methylene chloride content) = 0.7%]. Assuming that ethanol acts as a strong proton donor and/or acceptor and methylene chloride rather as a strong dipole, it can be inferred that the chiral recognition process involves hydrogen bonding rather than dipole stacking.

From the structure of APA methyl esters, three potential sites of interaction are evidenced (Fig. 5): the aromatic ring suitable for a π–π interaction, the ethereal oxygen atom (proton acceptor) and the ester function, which may be considered rather as a proton-acceptor site than as a dipole.

According to the above-mentioned statements, a π–π interaction and hydrogen bonding are involved in the chiral recognition. It can therefore be inferred that a 3,5-dinitrophenyl moiety and the proton-donor sites (amidic and hydroxyl proton) of the CSP are involved in the chiral recognition process.

Resolution of MCPP BOE ester

The second aspect of this work was the enantiomeric separation of MCPP BOE ester in order to determine its enantiomeric excess in a commercially available formulation. According to the above-mentioned chromatographic data, this separation should have been carried out using methylene chloride. Unfortunately, the formulation contains a second active constituent of high polarity, which is hardly eluted with methylene chloride. Consequently, the separation was better achieved using ethanol as polar modifier (0.125%, flow-rate 2 ml/min). The results obtained were $k'_2 = 6.87$, $\alpha = 1.34$ and $R_s = 3.1$. The complete separation is shown in the Fig. 6. The various additives (eluted within 4 min) and the second active constituent (eluted at 22 min) do not interfere with CMPP BOE ester enantiomers (eluted at 6 and 8 min).

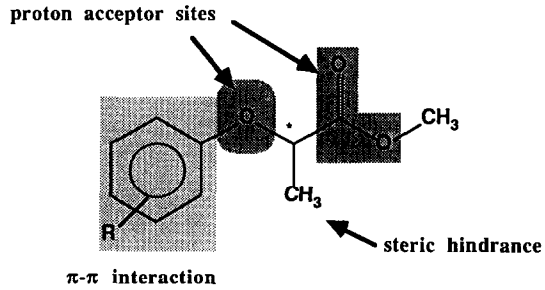


Fig. 5. Potential sites of interaction of APA methyl esters.

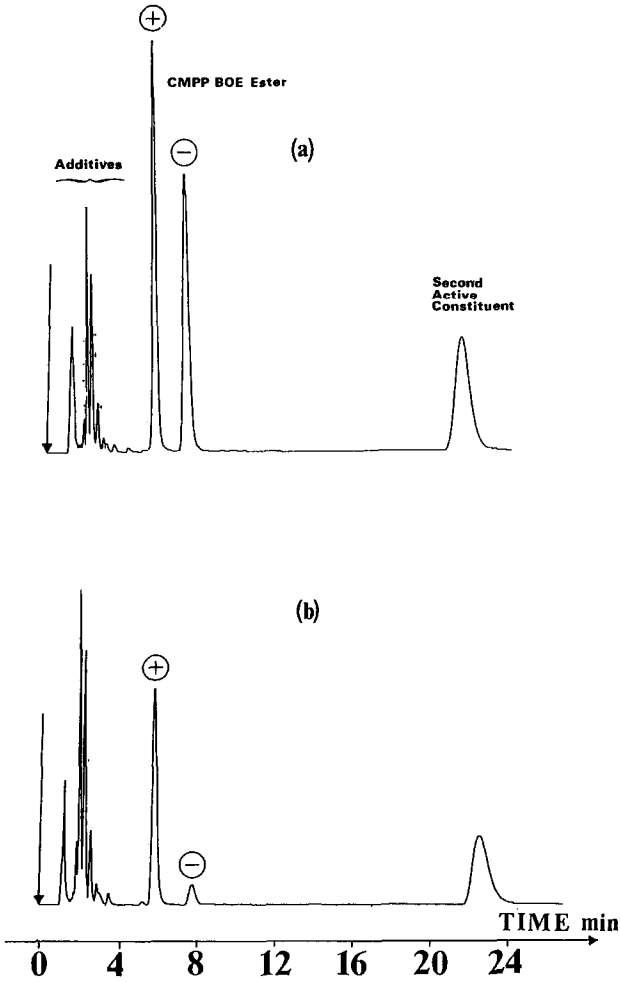


Fig. 6. Determination of the enantiomeric excess (e.e.) in a CMPP BOE ester formulation using a DACH-DNB CSP. Mobile phase, *n*-hexane–ethanol (99.875:0.125, v/v); flow-rate, 2 ml/min; room temperature; UV detection at 254 nm. (a) Racemic mixture; (b) e.e. = 75.4%.

CONCLUSION

The ability of the DACH-DNB CSP to resolve APA esters has been demonstrated. The selectivity values and resolution factors afforded by this CSP were higher than those reported previously for either ionic or covalent DNBPG [2,4]. We consider that the scope of application of this CSP is complementary to those of DNPG and CSPs derived from tyrosine. In fact, the DACH-DNB CSP affords a high enantioselectivity towards small molecules which are not or only poorly resolved on other Pirkle-type π-acid CSPs.

The chiral recognition mechanism involves a π–π interaction and hydrogen bondings. Enantioselectivity was found to be very sensitive to the electronegativity and

the position of the aromatic ring substituents when using ethanol and also to the polar modifier content in the mobile phase.

The enantiomeric separation of MCPP BOE ester was performed using a DACH-DNB CSP ($R_s = 3.1$). This method allows the determination of its enantiomeric excess in commercially available optically active formulations, such as those reported previously by Blessington and Crabb [2].

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