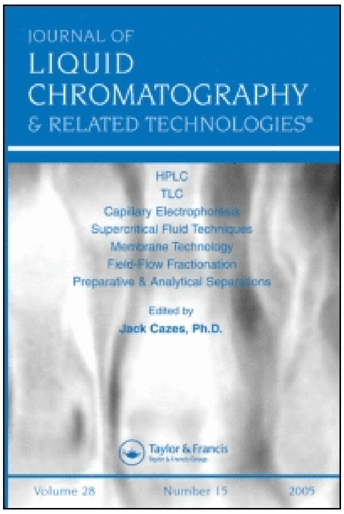

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CHIRAL RECOGNITION OF ALKYL 2-ARYLOXYPROPIONATES BY HPLC

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ABSTRACT

Chiral resolution of a series of antiphlogistic methyl and ethyl 2-aryloxypropionates on R-DNBPG and S-DNBL analytical columns was attempted. Most of the resolved enantiomers were eluted in a very short time on both CSPs and the α and K' values of the chromatographic separations performed by R-DNBPG phase were generally better than those using S-DNBL.
The elution order of the compounds was determined: the S isomer of all esters was eluted last from the R-DNBPG and viceversa from the S-DNBL column.
The role of the substituents on the chiral resolution was also elucidated. It was hypothesized that the chiral interactions between the solutes and the CSPs were more influenced by the electronic features than by their steric hindrance.
Finally, the chiral recognition mechanism which permitted resolution of the enantiomers was individuated.

INTRODUCTION

Our previous research (1,4) focused on the synthesis, chiral resolution of racemic mixtures and configurational assignment of enantiomers of a series of 2-aryloxypropionyl derivatives, which are biologically active as antinflammatory, analgesic agents and as plant growth regulators (5-7). Some of these compounds can also be structurally related to chiral analogs of chlofibrate whose pharmacological activity has been already investigated (8). Furthermore, it was pointed out that the interaction of similar drugs with biological receptors is stereoselective (9-12).

To obtain the chiral resolution of the racemic compounds, chromatography on chiral stationary phases had been also performed. The enantiomers of some 2-aryloxypropionic N-(1-naphthyl)methyl amides had been resolved by HPLC on Pirkle type phases, *i.e.* CSPs of R - N - (3,5 - dinitrobenzoyl)- phenylglycine (R-DNBPG) and S -N-(3,5-dinitrobenzoyl)-leucine (S-DNBL), covalently bound to SiO_2 (3). The resolution of a more numerous series of 2-aryloxypropionic acids and their methyl and ethyl esters (partially corresponding to the amidic derivatives) had also been accomplished on a column of cellulose tris - (3,5-dimethylphenylcarbamate) Chiralcel OD (4).

Chromatographic separation of 2-aryloxypropionyl esters by Chiralcel OD has been shown to be better than separation of the amides using R-DNBPG and S-DNBL columns. As the Chiralcel OD phase is very expensive, we wanted to verify whether chiral resolution of the esters could also be accomplished on Pirkle's CSPs. If so, this could permit the enantiomeric resolution of the racemates on a preparative scale with the added advantage that the esters can be hydrolyzed under milder conditions than those needed for the amides to furnish the acids without racemization. Then we experimented the resolution of the above-mentioned methyl and ethyl esters on Pirkle's CSPs.

The analyzed compounds, apart from **26**, bear one or two substituents in the 2, 3 and 4 position of the aryloxylic portion directly linked to the chiral center. These substituents were selected to introduce atoms or groups of various dimensions and with electron-donating or electron-withdrawing properties, in order to verify whether their size, chemical features or position on the phenyl ring influence the chromatographic discrimination process. In this context, the unsubstituted compound **26** was also analyzed.

By critically evaluating the experimental results and studying molecular models of the solute-CSP interactions, we also investigated the relationship between the configuration and elution order of the enantiomers to determine a rationale for the chiral recognition mechanism.

MATERIALS AND METHODS

Materials

All racemates and optical antipodes of methyl and ethyl esters were prepared as previously reported (4).

Hexane and 2-propanol (HPLC grade) were purchased from Farmitalia Carlo Erba. All other chemicals and solvents were of analytical-reagent grade.

Methods

Melting points were determined with a Buchi apparatus and are uncorrected.

The elemental analyses were carried out with a Perkin-Elmer 240 C,H,N analyzer and were within ± 0.4 % of the theoretical values.

$[\alpha]_D$ measurements were made with a Perkin-Elmer 141 M photoelectric polarimeter at 22° C.

The chromatographic analyses were performed using a Gilson model 303 isocratic pump, equipped with Holochrome HM variable wavelength detector, and Reodyne model 7125 injection valve. Experimental data were analyzed with the Gilson 715 HPLC software.Analytical columns (250 x 4.6 mm, 5 μm) of covalently bound R-DNBPG and S-DNBL phases were used. The analysis of any compound was performed on both the racemate and the R enantiomer to determine the elution order of the antipodes.

Several mixtures of hexane or iso-octane, as the main apolar component, and lower amounts of ethanol or 2-propanol were tested as eluting systems; 0.25 % 2-propanol in hexane was found to be the most suitable mobile phase, at a flow rate of 1 ml/min. UV detection at 260 nm was used.

Tables 1 and 2 report the capacity factors K'_1 and K'_2 of the first and second eluted enantiomer, the selectivity factor α, the resolution factor R_s and the elution order of the solutes.

Methyl esters of methyl-, chloro- and nitro-substituted compounds had been already examined,on an R-DNBPG column by Demoncour and Azerad (13) and their results are, in general, consistent with our findings.

TABLE 1 (part I)

Chromatographic Resolution of Methyl 2-Aryloxypropionates on R-DNBPG (upper values) and S-DNBL (lower values) CSPs.

Comp	Aryl	K'$_1$	K'$_2$	α	R$_s$	Last eluted
1 a	2-$CH_3C_6H_4$	0.88 0.94	1.14 1.03	1.29 1.10	1.03 0.84	S R
2 a	3-$CH_3C_6H_4$	1.41 1.36	1.58 1.47	1.12 1.08	1.06 0.78	S R
3 a	4-$CH_3C_6H_4$	1.55 1.50	1.71 1.60	1.10 1.07	1.19 0.60	S R
4 a	2-$C_2H_5C_6H_4$	0.80 0.80	0.90 0.89	1.12 1.12	0.95 0.95	S R
5 a	3-$C_2H_5C_6H_4$	1.19 1.26	1.31 1.37	1.10 1.09	0.94 0.88	S R
6 a	4-$C_2H_5C_6H_4$	1.32 1.25	1.47 1.34	1.11 1.07	1.12 0.68	S R
7 a	2,3-$(CH_3)_2C_6H_3$	1.11 1.09	1.30 1.24	1.17 1.13	1.56 1.06	S R
8 a	2,4-$(CH_3)_2C_6H_3$	1.06 0.97	1.25 1.10	1.18 1.13	1.23 0.99	S R
9 a	2,5-$(CH_3)_2C_6H_3$	1.00 0.95	1.20 1.06	1.20 1.12	1.69 0.88	S R
10 a	2,6-$(CH_3)_2C_6H_3$	0.97 1.02		1.00 1.00	— —	— —
11 a	3,4-$(CH_3)_2C_6H_3$	1.73 1.90	1.99 2.07	1.15 1.09	1.01 0.78	S R
12 a	2-ClC_6H_4	1.57 1.41	1.68 1.52	1.07 1.08	0.86 0.97	S R
13 a	3-ClC_6H_4	1.22 1.09	1.30 1.14	1.07 1.05	0.75 0.64	S R

TABLE 1 (part II)

Chromatographic Resolution of Methyl 2-Aryloxypropionates on R-DNBPG (upper values) and S-DNBL (lower values) CSPs.

Comp	Aryl	K'$_1$	K'$_2$	α	R$_s$	Last eluted
14 a	4-ClC_6H_4	1.31 1.19	1.39	1.06 1.00	0.69 —	S —
15 a	2-$NO_2C_6H_4$	8.19 9.11	8.34 9.32	1.02 1.02	0.52 0.53	S R
16 a	3-$NO_2C_6H_4$	4.67 4.52	4.81	1.03 1.00	0.70 —	S —
17 a	4-$NO_2C_6H_4$	7.43 6.27	7.58	1.02 1.00	0.50 —	S —
18 a	3-$(CH_3CO)C_6H_4$	12.23 10.00	12.95 10.26	1.06 1.03	0.90 0.44	S R
19 a	3-$(C_6H_5CO)C_6H_4$	13.24 9.38	13.79 9.65	1.04 1.03	0.81 0.72	S R
20 a	2-CH_3-3-(4-$ClC_6H_4CO)C_6H_3$	7.88 5.73	8.63 6.27	1.10 1.09	1.12 1.16	S R
21 a	4-$(C_6H_5)C_6H_4$	2.88 2.29	3.15 2.40	1.09 1.05	1.14 0.80	S R
22 a	4-(2,4-$F_2C_6H_3)C_6H_4$	2.54 1.98	2.71 2.09	1.08 1.06	1.03 0.86	S R
23 a	4-$(C_6H_5CH_2)C_6H_4$	2.35 1.85	2.50 1.96	1.06 1.06	0.79 0.68	S R
24 a	4-$(C_6H_5CO)C_6H_4$	17.69 14.46	18.86 14.87	1.07 1.03	1.05 0.70	S R
25 a	4-$(C_6H_5O)C_6H_4$	2.50 2.02	2.66 2.15	1.06 1.06	0.84 0.87	S R
26 a	C_6H_5	1.50 1.55	1.59 1.62	1.06 1.05	0.79 0.35	S R

TABLE 2 (part I)

Chromatographic Resolution of Ethyl 2-Aryloxypropionates on R-DNBPG (upper values) and S-DNBL (lower values) CSPs.

Comp	Aryl	K'$_1$	K'$_2$	α	R_s	Last eluted
1 b	2-$CH_3C_6H_4$	2.44 1.99	2.75 2.11	1.13 1.06	1.37 0.51	S R
2 b	3-$CH_3C_6H_4$	2.19 3.06	2.42 3.13	1.10 1.02	1.00 0.21	S R
3 b	4-$CH_3C_6H_4$	1.23 1.17	1.36 1.22	1.10 1.04	0.99 0.54	S R
4 b	2-$C_2H_5C_6H_4$	0.47 1.90	0.55 2.09	1.16 1.10	1.00 0.97	S R
5 b	3-$C_2H_5C_6H_4$	0.76 2.16	0.85 2.27	1.11 1.05	1.12 0.66	S R
6 b	4-$C_2H_5C_6H_4$	2.73 3.67	2.92 3.81	1.07 1.04	0.89 0.53	S R
7 b	2,3-$(CH_3)_2C_6H_3$	1.09 1.07	1.29 1.17	1.18 1.09	1.74 0.81	S R
8 b	2,4-$(CH_3)_2C_6H_3$	0.98 1.13	1.15 1.24	1.17 1.10	1.20 0.79	S R
9 b	2,5-$(CH_3)_2C_6H_3$	2.01 2.58	2.33 2.68	1.16 1.04	1.79 0.38	S R
10 b	2,6-$(CH_3)_2C_6H_3$	0.65 1.14	0.68	1.05 1.00	0.59 —	S —
11 b	3,4-$(CH_3)_2C_6H_3$	1.43 0.79	1.65 0.84	1.15 1.06	1.71 0.56	S R
12 b	2-ClC_6H_4	1.57 1.60	1.68 1.70	1.07 1.07	0.86 0.77	S R
13 b	3-ClC_6H_4	1.11 1.07		1.00 1.00	— —	— —

TABLE 2 (part II)

Chromatographic Resolution of Ethyl 2-Aryloxypropionates on R-DNBPG (upper values) and S-DNBL (lower values) CSPs.

Comp	Aryl	K'$_1$	K'$_2$	α	R$_s$	Last eluted
14 b	4-ClC_6H_4	1.04		1.00	---	---
		2.65		1.00	---	---
15 b	2-$NO_2C_6H_4$	8.93		1.00	---	---
		8.54		1.00	---	---
16 b	3-$NO_2C_6H_4$	3.89		1.00	---	---
		2.98		1.00	---	---
17 b	4-$NO_2C_6H_4$	5.43		1.00	---	---
		5.10		1.00	---	---
18 b	3-$(CH_3CO)C_6H_4$	26.16	27.62	1.06	0.90	S
		8.43		1.00	---	---
19 b	3-$(C_6H_5CO)C_6H_4$	10.84	11.30	1.04	0.91	S
		8.30	8.46	1.02	0.57	R
20 b	2-CH_3-3-(4-$ClC_6H_4CO)C_6H_3$	6.15	6.72	1.09	1.71	S
		4.53	4.84	1.07	1.35	R
21 b	4-$(C_6H_5)C_6H_4$	3.15	3.46	1.10	1.04	S
		2.96		1.00	---	---
22 b	4-(2,4-$F_2C_6H_3)C_6H_4$	1.80	1.93	1.07	1.01	S
		1.53		1.00	---	---
23b	4-$(C_6H_5CH_2)C_6H_4$	2.68	2.88	1.07	0.86	S
		2.53	2.60	1.03	0.34	R
24 b	4-$(C_6H_5CO)C_6H_4$	18.32	18.93	1.03	0.81	S
		12.58	12.77	1.02	0.38	R
25 b	4-$(C_6H_5O)C_6H_4$	2.60	2.80	1.08	1.03	S
		2.36	2.42	1.03	0.48	R
26 b	C_6H_5	1.23	1.31	1.07	0.83	S
		1.35		1.00	---	---

RESULTS AND DISCUSSION

Chromatographic separation of the racemic compounds and R enriched samples (arising from the synthesis of the optically active compounds) was performed to determine the correct elution order of the enantiomers.

As expected , the chromatographic chiral resolution of ethyl and methyl esters on both R-DNBPG and S-DNBL phases was very similar. Nearly all the esters appeared to be more suitable derivatives than the corresponding amides (3) for the chiral interaction with CSPs. This is because in general they show comparable α and R_s values, but shorter elution times. K' values of both ethyl and methyl esters are much lower than those of the amides, even if the eluting system contains much less 2-propanol (0.25 % *vs* 5 or 10 %), normally used for avoiding excessively strong interactions of solutes with stationary phases by means of hydrogen bonding. Apart from the sites involved in the mechanism of chiral discrimination by hydrogen bonding, on the CSP there are other sites which can give this type of interaction; the amount of 2-propanol must thus be enough to saturate these sites.

Methyl and ethyl esters showed better values of α and R_s parameters on R-DNBPG than on S-DNBL phase, in contrast to the amides.

On comparison of the elution order of the esters and amides it appeared that, while the S enantiomer of almost all amides was eluted last on both the CSPs, the S enantiomer of all methyl and ethyl esters was more retained than the R enantiomer on R-DNBPG and vice versa on S-DNBL phase.

We had already observed that the enantiomers of the amides, bearing a second carbonyl group on the aryloxylic portion (acetyl or benzoyl derivatives), were eluted with longer retention times and were less resolved than the others. Higher K' values can be also observed for the corresponding esters **18a-20a**, **24a** and **18b-20b**, **24b**. These results could signify that these compounds strongly interact with the stationary phases by means of the acetyl or benzoyl moieties that can give rise to hydrogen bonds or dipole-dipole bonding which do not involve the chirality of the interaction between the molecules and the CSPs.

The influence that the substituents of the aryloxylic portion have on the extent of the chiral resolution was pointed out. We observed that the presence of electron-donating methyl and ethyl groups produced an increase in α and R_s values. This is in agreement with the assumption that the strength of the π–π interaction of the solute with the π acidic moiety of CSP (dinitrobenzoyl group) is increased because of the π basic property of the methyl or ethyl substituted aryl group.

In contrast, compounds bearing electron-donating groups with greater steric hindrance (*e. g.* phenyl, benzyl, phenoxyl), as well as the unsubstituted derivative and derivatives with electron-withdrawing substituents were less resolved chromatographically.

The poor or complete lack of resolution of the compounds with electron-withdrawing substituents indicate that the π–π interaction between their aryloxylic portion and the dinitrobenzoyl group of CSP is unlikely because the π electrons of the substituted phenyl ring are less available. The higher K' values of the nitro derivatives show the presence of other interactions, which are not determinant for chiral separation.

It was also observed that of the compounds with electron-donating groups, the esters bearing a second aromatic ring (phenyl, benzyl, phenoxyl), showed higher K' values with respect to methyl, dimethyl or ethyl derivatives. This could signify a stronger interaction with the stationary phase because of the higher stability of the solute-CSP complex when a second aromatic ring was present. In fact, another π–π interaction, which does not involve the chirality of the molecule and delays the elution of the solutes through the column, can take place.

Chiral Discrimination Mechanism

Apart from the analysis of the experimental results, we also investigated interactions of the esters with the chiral phases using molecular models, which enabled us to recognize more probable interaction sites of both the CSPs and solutes.

We assumed (Figg. 1 and 2) that in the conformation by which the CSPs moiety interacts with the solutes, the dinitrobenzoyl carbonyl oxygen is near the methyne hydrogen, presumably for reasons of carbinyl hydrogen bonding (as already proposed by Pirkle *et al.* (14)). Further hydrogen bonding is also possible between the carbonyl next to the chiral center and the aminoacidic NH group.

Figures 1 and 2 show that the possible interactions for the more retained enantiomer (S for R-DNBPG and R for S-DNBL) are:

a) dipole-dipole stacking between the carbonyl of 3,5-dinitrobenzoyl group of the stationary phase and the carbonyl of the propionyl group of the solutes;

b) π–π interactions of the dinitrobenzoyl group of CSPs and the aryloxyl portion of the solutes;

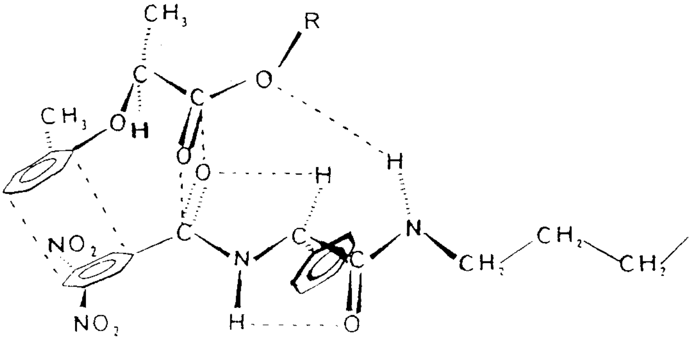

FIGURE 1. Interactions of enantiomer S-1 with R-DNBPG phase

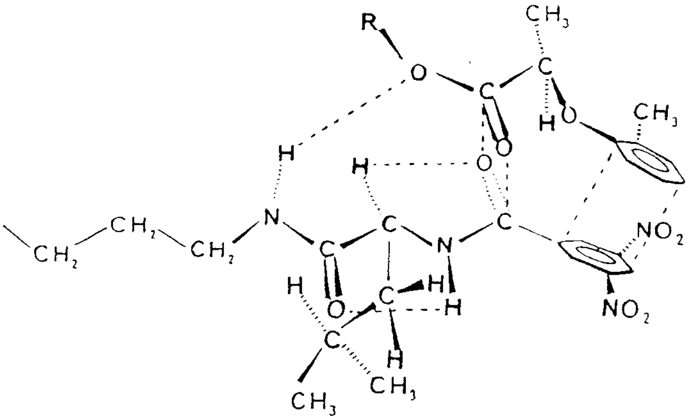

FIGURE 2. Interactions of enantiomer R-1 with S-DNBL phase

c) hydrogen bonding between the carbamic NH group of CSP and the oxygen of the ester group of the solutes.

All these interactions are possible depending on the configuration of the solutes. They can thus give rise to chiral discrimination because the methyl group, directly attached to the asymmetric carbon atom, prevents optimum alignment of the solute and CSP for less retained enantiomers whose diastereomeric complexes with CSPs have lower stability.

This discrimination mechanism agrees with experimental data.

For the unresolved esters, other interactions (also those already mentioned for the nitro-derivatives), can obviously prevent the chiral interaction.

CONCLUSIONS

We experimented chiral resolution of methyl and ethyl esters of twenty-six 2-aryloxypropionic acids and, for numerous compounds, this was accomplished in a very short time. Compounds bearing a carbonyl group at the aryloxylic moiety showed highest K' values.

The degree of chiral resolution did not appear to depend on the steric hindrance of the substituents, but rather on their electronic features. Furthermore, the electron-donating properties of methyl and ethyl groups improved the resolution, as shown by the comparison of the α and R_S values of the methyl and ethyl substituted compounds, those of the unsubstituted **26** and of the compounds with electron-withdrawing substituents.

Critical analysis of the experimental results and the study of the solute-CSP interactions, by means of molecular models, allowed us to establish the chiral recognition mechanisms which lead to the enantiomeric separation of the examined compounds.

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REFERENCES

1. Azzolina O., Vercesi D., Ghislandi V., *Il Farmaco Ed. Sc.*, **40**, 145; 1985.
2. Azzolina O., Vercesi D., Ghislandi V., *Il Farmaco Ed. Sc.*, **43**, 469; 1988.
3. Azzolina O., Ghislandi V., *Il Farmaco*, **48**, 713 ; 1993.

4. Azzolina O., Collina S., Ghislandi V., *Il Farmaco*, **48**, 1401 ; 1993.

5. Brit. Pat. 916,242 (Jan. 23, 1963, Appl.Jan. 7 and Sept. 16, 1959) : Boots Pure Drug Co., Ltd. (Designated inventors : Lewis H R., Housley J.R., Richards H.C., Nicholson J.S., Baker M.W., Adams S.S.).

6. Marzo A., Quadro G., Bruno G., Treffner E., Reali F. L., Borrelli G., Stradi R., *Arzneim.-Forsch.*, **33**, 198; 1983.

7. Worthing C.R., **The Pesticide Manual**, BCPC Publications, London, 6th ed., 329; 1979.

8. Witiak D.T., Ho C.-L.T., Hackney R.E., Connor W.E., *J. Med. Chem.*, **11**, 1086; 1968.

9. Bettoni G., Bryant S.H., Conte Camerino D., Feller D.R., Grasso G., Kamanna V.S., Loiodice F., Mambrini M., Newmann H.A.I., Romstedt K.J., Tortorella V., Witiak D.T., *Actual Chim. Ther.*, **15**, 125; 1988.

10. Feller D.R., Kamanna V.S., Newmann H.A.I., Romstedt K.J., Witiak D.T., Bettoni G., Bryant S.H., Conte Camerino D., Loiodice F., Tortorella V. , *J. Med. Chem.*, **30**, 1265; 1987.

11. Bettoni G., Loiodice F., Tortorella V., Conte Camerino D., Mambrini M., Ferrannini E., Bryant S.H., *J. Med. Chem.*, **30**, 1267; 1987.

12. Lien E.J., Rodrigues de Miranda J.F., Ariens *J., Mol. Pharmacol.,* **12**, 598; 1976.

13.Dernoncour R., Azerad R., *J. Chromatography* ,**410**, 355; 1987.

14. Pirkle W. H., House D. W., Finn J. M., *J. Chromatography*, **192**, 143; 1980.

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