Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 9, pp. 2577-2584, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00331-X

Separation of the Enantiomers of Anticonvulsant Tricyclic Pyrroloimidazolones by Enantioselective HPLC. A Chiral Recognition Model and a Chiroptical Study

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Abstract: The enantiomers of several pyrrolobenzimidazolone and pyrroloimidazopyridine derivatives have been successfully resolved by HPLC on a Whelk-O 1 Chiral Stationary Phase superior to other CSPs. A chiral recognition model explained the substituent effects on the enantioselectivity and afforded correlation of the elution order of the enantiomers to their absolute configuration. The exciton coupling method was applied to the CD spectra of the isolated enantiomers to confirm their absolute configuration.

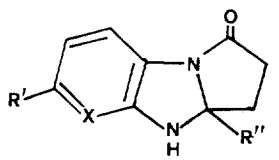
The therapeutic efficacy of marketed antiepilectic drugs cannot be deemed satisfactory and moreover they possess a broad range of undesiderable side effects. In order to obtain anticonvulsant agents with more selective activity and lower toxicity, we synthesized a series of 2,3,3a,4-tetrahydro-1*H*-pyrrolo[1,2-a] benzimidazol-1-ones[1] and analogous 7,8,8a,9-tetrahydro-6*H*-pyrrolo[1',2':1,2]imidazo[4,5-b]pyridin-6-ones[2] and most of them showed an anticonvulsant effect better than that of valproate, a commonly used anticonvulsant drug.[1-4] However, the pharmacological evaluations previously reported refer only to racemic compounds, but a different degree of anticonvulsant activity or specificity of action of the two isolated enantiomers is to be expected. This prompted us to search for a direct method of enantiomeric separation. Indeed, recent guidance has been given for a toxicity and activity testing during the development process of chiral drugs[5] because of the importance, previously underestimated,[6] of the enantioselectivity of chiral drugs with respect to the receptor sites. In this respect, biological *in vivo* enantioselectivity of anticonvulsant cyclic amides used in therapeutic preparations were reported[7] and resolution of enantiomeric succinimides was achieved.[8]

Beside the pharmacological aspect, another point of interest was the possible three-point interaction of these substrates with the Whelk-O 1 phase, a recently developed chiral stationary phase (CSP), working particularly well with chiral compounds possessing "complementary functionality" needed for chiral recognition by this CSP.[9] We report here a good separation of the enantiomeric pair of these candidate drugs with this CSP that is far superior to other CSPs. Indeed, a chiral recognition model explained the substituent effects on the enantioselectivity and gave an hypothesis on the absolute configuration of the eluted enantiomers. A strong Cotton effect in the CD spectra of the single enantiomers permitted the application of the exciton coupling method to confirm the absolute configuration of the isolated enantiomers.

Results and Discussion

The chromatographic resolution of compounds **1-13** was mainly examined using the Whelk-O 1

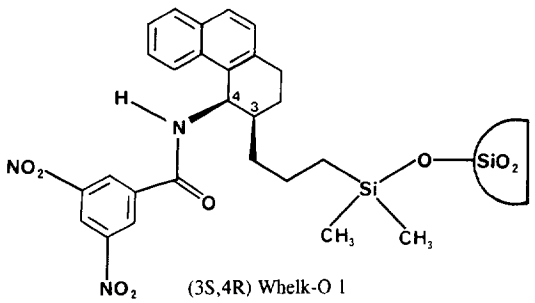
CSP as this was deemed the most suitable phase due to the "complementary functionality" approach betweeen the CSP and the analyte (see below).



R'=H, Cl, NO_2 X=CH, N

R"=CH_3, C_6H_5, 4-F-C_6H_4, 4-Cl-C_6H_4

Compounds **1**-**13**: various combinations of X, R', R".



(3S,4R) Whelk-O 1

Table 1 shows the chromatographic results. Enantioselectivity (α) ranges from 1.90 for compound **4** to 1.25 for compound **13**, using as eluent *n*-hexane/2-propanol 7:3. These are good values that are still improved for compounds **9**-**12**, which contain a basic pyridine group, by the addition of a small amount of diethylamine (DEA) in the eluent. The beneficial effect of DEA is more evident on the resolution factor (R_S), as shown for example by the results obtained for compound **12**, where α and R_S improve from 1.51 and 2.0 to 1.59 and 2.9 respectively, using as eluent *n*-hexane/2-propanol 7:3, as shown also in Figure 1. The presence of DEA in the mobile phase suppresses also the tailing of the enantiomeric peaks.

Table 1. HPLC Resolution of Enantiomeric Compounds **1**-**13** on (3S,4R) Whelk O1 CSP

Comp.	X[a]	R'[a]	R"[a]	k'[b,c]	α	R_S
1	CH	H	CH_3	1.61	1.48	2.5
2	CH	H	C_6H_5	1.66	1.69	4.2
3	CH	H	4-F-C_6H_4	1.29	1.83	4.0
4	CH	H	4-Cl-C_6H_4	1.37	1.90	4.5
5	CH	Cl	CH_3	1.38	1.44	2.1
6	CH	Cl	C_6H_5	1.34	1.60	3.1
7	CH	Cl	4-F-C_6H_4	1.18	1.72	3.3
8	CH	Cl	4-Cl-C_6H_4	1.34	1.78	4.3
9	N	H	CH_3	3.27 2.36[d]	1.28 1.33	1.0 1.4
10	N	H	C_6H_4	2.34 1.90[d]	1.38 1.45	1.5 2.2
11	N	H	4-F-C_6H_4	1.93 1.70[d]	1.49 1.55	1.6 2.5
12	N	H	4-Cl-C_6H_4	2.06 1.73[d]	1.51 1.59	2.0 2.9
13	CH	NO_2	CH_3	2.56	1.25	1.8

[a] According to the general formula in the text. [b] Capacity factors of the first eluted enantiomer.
[c] *n*-hexane/2 propanol 7:3 at a flow rate of 1 ml/min., t_0=3.10 min.. [d] 2-Propanol doped with 0.5 % of diethylamine.

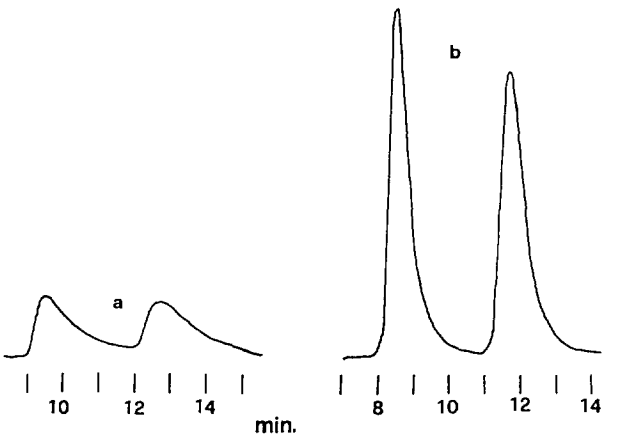


Fig.1 HPLC resolution of **12** on Whelk-O 1 at a flow rate of 1 ml/min with mobile phase **(a)** *n*-hexane- 2-propanol 7:3 and **(b)** *n*-hexane/2-propanol doped with 0.5 % of diethylamine (7:3).

The most important observation from the results in Table 1 is the beneficial effect on the extent of the enantioselectivity of the π-character of the R" group and of the dipole moment of the 4-substituent in R" (1.47 and 1.59 D for 4-F and 4-Cl respectively).[10] This is shown by comparing the α values of compounds **1-4**, **5-8** and **9-12** respectively. The relationship between the substituents and the enantioselectivity is indicated in Figure 2 a and b, where logα is reported as a function of varying R" substituents with respect to logα of reference compounds.

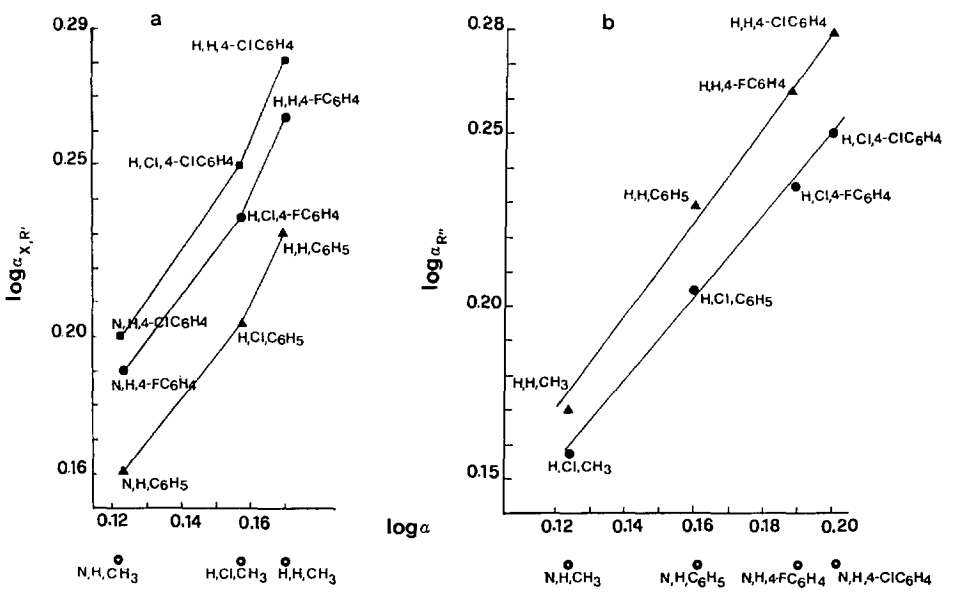


Fig.2 The curves represent **(a)** the log $\alpha_{X,R'}$ where X and R' are the varying substituents in the general formula; **(b)** the log $\alpha_{R"}$ where R" is the varying substituent in the general formula. Log α of reference compounds, where CH_3 is the unchanged R" substituent **(a)** and N and H respectively are the unchanged X and R' substituents **(b)**, are reported in the abscissa.

A rationalization of this trend resides in the model for the enantiodifferentiation which we propose. The CSP used by us has been recently developed by Pirkle and Welch [11,12] and it proved to be a highly enantioselective selector of wide applicability.[9] It has a cleft and inside it one enantiomer is preferentially

bound. As shown in the formula of the CSP reported above, the cleft consists of π-acidic and π-basic aromatic systems held almost perpendicular to each other.

Taking into account the most stable conformations of the CSP[13] and of the analyte,[14] (both obtained from X-ray diffraction analysis) we built a chiral recognition Dreiding model between them. The simultaneous π-donor-acceptor interactions and dipole stacking interactions between the selector and the most retained enantiomer of the analyte are shown in Figure 3. The three chiral interactions are 1) the face-to-face π-π interaction between π-acid dinitrobenzamide group of the CSP and the benzimidazole system of the analyte, 2) the face-to-face π-π interaction between the π-basic naphthyl group of the CSP and the phenyl group of the analyte, 3) the antiparallel dipole stacking between the carboxamide group of the CSP and the lactam moiety of the analyte. This third interaction is very strong. In fact, the dipole moment of an amide group is very high (3.84 D) and its direction forms an angle of almost 40° with the C-N bond,[15] as indicated in Figure 3. This model explains also the effect of structural changes in X, R' and R" on the extent of enantioselectivity. Lower α values of compounds **9**-**12** are explained by the minor interaction 1, due to the pyridine π-withdrawing group. Better α values of R"-halogenated compounds **3**, **4**, **7**, and **8** are explained by the better interaction 2, due to a more π-acid aryl group. Analogously, lower α values of compounds **1**, **5**, **9** and **13** are explained by the weaker interaction 2, due only to the R" electron-releasing methyl group. The lowest α value in Table 1 is found for compound **13**, where also interaction 1 is unfavorable due to the R' electron-withdrawing group.

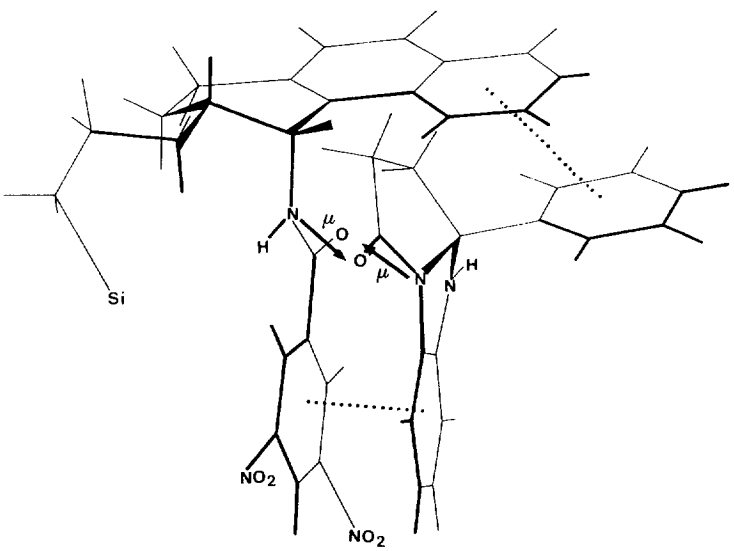


Fig.3 Molecular model representation of the diastereomeric adsorbate between (3S,4R) Whelk-O 1 phase and (S) compound **2**.

The enantiodifferentiation arises from the presence or not of interaction 3. In fact the alternate enantiomer, not depicted in Figure 3, cannot form antiparallel dipole stacking. The absolute configuration of the Whelk-O 1 phase used by us is 3S,4R as depicted in Figure 3 and the most strongly retained enantiomer was thus the S form as shown in Figure 3.

We should remark that the absolute configuration of the Whelk-O 1 phase used by us does not correspond to that indicated in the label of the column (R,R).[16] This is simply due to an inversion of priority in the Cahn-Ingold-Prelog stereochemistry system. In fact the original (R, R) phase had a longer

methylenic tether before reaching silicon[11] with respect to the three methylene tether as in Whelk-O 1.

The influence of the complementary functionality and of the "spatial complementarity" on the enantioselectivity is particularly evident in three interactions between the Whelk-O 1 CSP and these pyrroloimidazolone derivatives. Both in fact possess an L-shaped structure. Table 2 reports the chiral resolution results of compounds **1-4**, using another Pirkle's CSP (DNBPG) and an helical polysaccaride CSP (Chiralcel OD). The enantioselectivity values are much less satisfactory with respect to those obtained using the rationally designed phase Whelk-O 1 and reported in Table 1. In one case (compound 1) no resolution was obtained at all, using both CSPs and also less polar mobile phases not reported in the Table. Superiority of the Whelk-O 1 phase is shown in Figure 4, where the striking difference in the enantioselectivity for compound **4** is reported for three investigated CSPs.

Table 2. Chiralcel OD and DNBPG CSPs Resolution of Compounds 1-4

Comp.	CSP[a]	k'[b,c]	α	R_S
1	A	0.36	NS[d]	
	B	0.74	NS	
2	A	0.34	1.56	1.1
	B	0.68	NS	
3	A	0.29	1.70	1.24
	B	0.66	NS	
4	A	0.32	1.73	1.2
	B	0.69	NS	

[a] Chiral Stationary Phases, A=Chiralcel OD and B=DNBPG. [b] Capacity factor of the first eluted enantiomer. [c] *n*-hexane/2-propanol 7:3 at a flow rate of 1 ml/min., t_0=3.34 min. and 3.40 min. respectively with A and B CSPs. [d] Not separated.

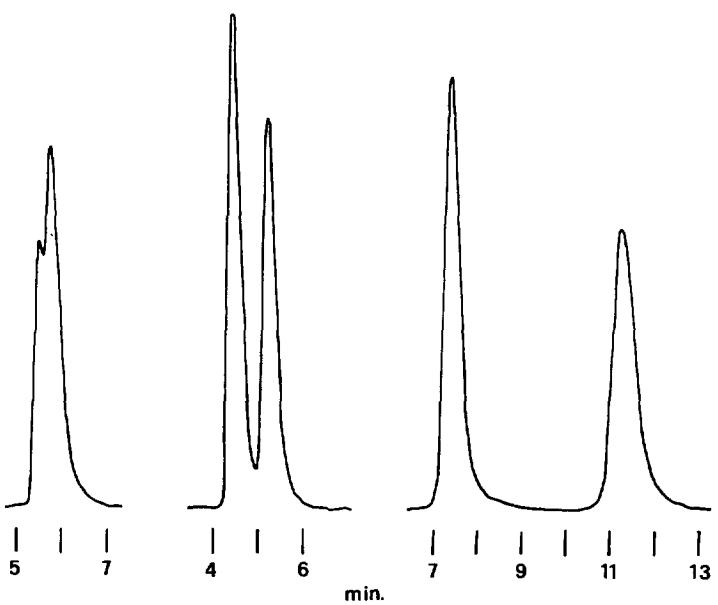


Fig.4 HPLC behaviour of the pair of enantiomers of **4** with three CSPs: (R) DNBPG, Chiralcel OD, Whelk-O 1. Mobile phase *n*-hexane/2-propanol 7:3 at flow 1 ml/min.

Further validation of this proposed configuration assignment is obtained from the chiroptical behaviour of the enantiomers of compounds **2** and **4**.

The excellent resolution factors obtained for these compounds afforded a semipreparative separation of their enantiomers by repeated 100 μl injections of racemic **2** and **4** and collection of the eluates from the chromatographic peaks. The CD spectra of both eluates for each compound were measured and they were mirror images of each other as shown in Figure 5 indicating that the two eluates from compound **2**, as well as those from compound **4**, are enantiomers. Analytical HPLC reruns of the eluates indicated an excess (ee) of 95% for the first peak and 98% for the second peak of compound **2**, and 97 % for the first peak and 100 % for the second peak of compound **4**. The UV spectra of the enantiomeric pairs were also identical. Compound **2** exhibited λ_{max} at 215 nm (ϵ=16800), at 252 nm (ϵ=4500) and at 309 nm (ϵ=3450). Compound **4** exhibited λ_{max} at 221 nm (ϵ=31700), at 252 nm (ϵ=7900) and at 309 nm (ϵ=5500). Inspection of the CD spectra of compounds **2** and **4** shows immediately remarkable Cotton effects (CE). In fact the CD split shows a positive CE ($\Delta\epsilon$ + 30 at 258 nm, $\Delta\epsilon$ - 48 at 214 nm) for the most retained enantiomer of compound **4**. Negative CE is instead observed for the less retained enantiomer. A similar situation is observed for compound **2**.

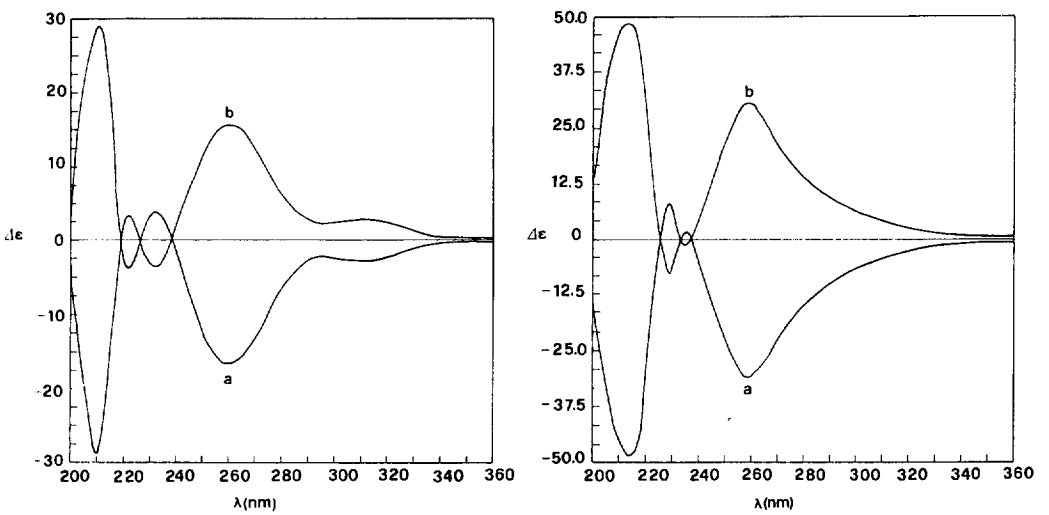


Fig. 5 CD spectra of the pair of enantiomers of compound **2** (left) and compound **4** (right) obtained from the first **(a)** and the second **(b)** HPLC eluted peaks.

The strong electric transition moments that interact spatially to give the CE effects are due to the π,π^* transition at 214 nm, that can be attributed to the amide bond with an additional contribution of aromatic chromophores, and to the π,π^* transition at 258 nm exclusively due to an aromatic chromophore. The fine structure of the CD spectra in the range 220-235 nm can be due to an additional coupling of other minor transitions, including n,π^* of the amide group.

The exciton chirality method is extremely versatile for establishing the absolute configuration from the knowledge of the absolute sense of twist between two chromophores.[17] The sign of the shorter frequency CD peak is related in fact to the sense of handedness of the interacting transition moments. The exciton coupling occurs also between two different chromophores,[18] and this is our case. The directions of the interacting transition moments of the lactam group and of the aryl group located on the stereogenic carbon

are roughly at 90° each other. The transition moment t_1 actually results from the summation of contributions from the arylamine and the arylamide chromophores. Thus the model shown in Figure 6 is built for the enantiomer possessing the positive CE chirality. This enantiomer results in having the S absolute configuration. This finding corroborates the chiral recognition model discussed above and is another example of the extreme usefulness of the exciton chirality method to determine the absolute configuration of a variety of compounds.

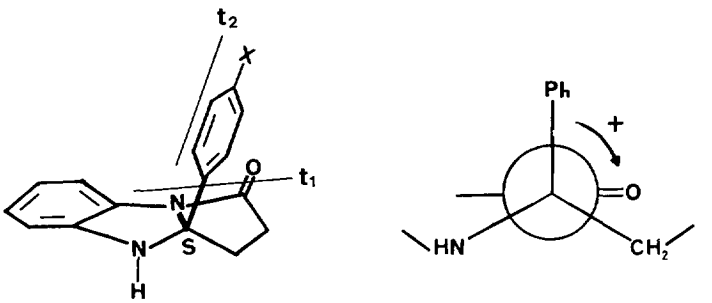


Fig. 6 The exciton chirality model, with the direction of the transition moments t_1 and t_2, obtained for the enantiomer of **2** and **4** possessing positive chirality.

Experimental

In previous papers some of us described the synthesis of compounds **1** and **2**,[14] compounds **3** and **4**,[1] compounds **5-8**, **13**[19] and compounds **9-12**.[2] The synthesis of compounds **1-8** was carried out by reacting 1,2-phenylendiamine or 4-chloro-1,2-phenylenediamine with the appropriate 3-acylpropionic acid in boiling toluene with azeotropic removal of water. Compounds **9-12** were synthesized analogously starting from 2,3-diaminopyridine as amine. The obtained products were isolated by flash chromatography. Various attempts to obtain single crystals of the individual enantiomers of **2** and **4**, obtained from chiral HPLC, for X-ray diffraction analysis in *n*-hexane/benzene and *n*-hexane/dichloromethane mixtures were unsuccessful.

The HPLC system consisted of a Varian 5060 liquid chromatograph with Valco sample loops, a Jasco Uvidec III UV spectrophotometric detector operating at 240 nm, and a Varian CDS 401 Data System or a Omniscribe Houston recorder for fraction collecting. CD spectra were recorded in ethanol on a Jasco 600 Spectropolarimeter. UV spectra were recorded in ethanol on a Beckman DU 650 Spectrophotometer.The mobile phases were HPLC-grade *n*-hexane/2-propanol mixtures. The columns (25 cm x 4,6 mm) were packed with Whelk-O 1 [4-(3,5-dinitrobenzamido)-tetrahydrophenanthrene] covalently bonded to 5 μm 3-propyl silica, (R)-DNBPG (N-3,5 dinitrobenzoylphenylglycine) covalently bonded to 5 μm aminopropylsilanized silica, both from Regis Chemical (Morton Grove, IL) and Chiralcel OD (Cellulose tris-3,5-dimethylphenylcarbamate) coated on 10 μm silica gel, from Daicel (Tokyo). A *caveat* about the stereochemistry nomenclature of the Whelk-O 1 column is given in the Discussion section. Column void time (t_0) was measured by injection of tri-*tert*-butylbenzene as a nonretained sample. Retention times were mean values of two replicate determinations. All separation were carried out at room temperature.

Acknowledgment. Financial support from the Ministero Università e Ricerca Scientifica e Tecnologica (MURST, Funds 40 %) to S. C. is gratefully acknowledged.

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(*Received in UK* 19 *June* 1996; *accepted* 25 *July* 1996)