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Liquid chromatographic separation of the enantiomers of antihistaminic 3,3′-di(1,3-thiazolidin-4-one) derivatives with two and four stereogenic centres

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Abstract

The enantiomers of anti-inflammatory and antihistaminic 3,3′-(1,2-ethanediyl)bis(2-aryl-1,3-thiazolidin-4-one) derivatives possessing two stereogenic centres were separated on Chiralcel OD stationary phase without derivatization. The *meso* form was also well separated from the enantiomers. The good resolution afforded a milligram-scale separation and subsequent measurement of the circular dichroism spectra of an enantiomeric pair. Addition of racemic α-mercaptopropionic acid to the N,N′-dibenzylideneethylenediamine yielded ten possible stereoisomers with four stereogenic centres. Two centres (2 and 2′) bear the same groups; the other two (5 and 5′) also bear the same groups, but these are different from the groups at 2 and 2′. In this situation four enantiomeric pairs and two *meso* forms exist; all of them were separated and identified using a Chiralpak AD column.

1. Introduction

3,3′-Di(1,3-thiazolidin-4-one) derivatives bearing an aryl group in positions 2 and 2′ (Fig. 1, R = H) were previously synthesized and investigated for their anti-inflammatory and antihistaminic properties [1,2]. Some of them, particularly those bearing an ethylenic chain between the 3- and 3′-nitrogen atoms and a 3-F or 3-Cl-phenyl group in position 2 and 2′, showed good anti-inflammatory and/or antihistaminic activity and also analgesic and antipyretic properties, their acute toxicity levels being lower than those of indomethacin and phenylbutazone used as reference compounds. Potency differences between racemic and *meso* isomers were often observed [2,3], the former usually being more active than the latter.

Since major differences in stereoselective ac-

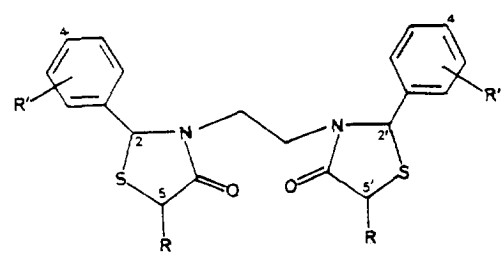

Fig. 1. Structures of compounds **1–16**: R = H, CH_3; R′ = 3-Cl, 3-Br, 3-F, 3-CF_3, 3,4-Cl_2, 3,4-$(OCH_3)_2$.

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tivities between the enantiomers of chiral antihistamines are well documented [4,5] and as a guide towards the pharmacokinetic and toxicological profiles of enantiomeric drugs has recently been issued [6], we decided to separate the enantiomers of the racemic compounds in order to submit single enantiomers to pharmacological evaluations.

This paper reports the enantiomeric resolution of several compounds with two stereogenic centres and one compound with four stereogenic centres due to an additional methyl in positions 5 and 5′ (Fig. 1, R = CH_3). The resolution was obtained without any derivatization using Chiralcel OD or, in one instance, Chiralpak AD as the stationary phase. The optimum mobile phase composition depended on the 2,2′ substituent group in the structure of the compound. The resolution of compound **5** (R′ = 3 − F, R = H) afforded a milligram-scale separation and measurement of the circular dichroism (CD) spectra of the individual enantiomers.

2. Experimental

Compounds **1–12** were available from previous studies [2,7]. The synthesis of the **13–16** is reported elsewhere [8]. The general procedure involves the addition of an aqueous solution of 1,2-diaminoethane to the appropriate aldehyde (0.5 molar ratio in ethanol) to obtain an N,N′-dibenzylideneethylendiamine that precipitates and is subsequently purified. To this compound, dissolved in toluene, an excess of mercaptoacetic acid (or racemic α-mercaptopropionic acid for the synthesis of **13–16**) is added and refluxed. After work-up, the title compounds are isolated.

The purity of **1–12** was checked by TLC (one distinct spot). Compounds **13–16**, all stereoisomers coming from a unique reaction, gave four distinct spots (see later). TLC was carried out on silica gel plates (Merck) using $CHCl_3$–Et_2O (9:1) as the eluent. Radial TLC was carried out by a Chromatotron apparatus (Harrison Research, Model 7924T) with silica gel 60P F_{254} gypsum-containing plates, by using light petroleum–Et_2O 9:1) as the eluent.

The HPLC system consisted of a Varian Model 5060 liquid chromatograph with Valco 10- or 50-μl sample loops, a Jasco Uvidec III UV spectrophotometric detector operating at 240 nm and a Varian CDS 401 data system or a Houston Omniscribe recorder for fraction collection. CD spectra were recorded on a Jasco Model 600 spectropolarimeter. The mobile phases were HPLC-grade *n*-hexane–2-propanol or *n*-hexane–dichloromethane mixtures as specified in the tables. As chiral phases a Chiralcel OD column (cellulose tris-3,5-dimethylphenylcarbamate) and a Chiralpak AD column (amylose tris-3,5-dimethylphenylcarbamate) coated on silica gel (each 25 cm × 0.46 cm I.D., particle size 10 μm), both from Daicel (Tokyo, Japan) were used. For normal-phase separations an Ultrasphere silica gel column from Beckman (15 cm × 2 mm I.D.) was used. Retention factors (k'), separation factors (α) and resolution (R_S) were calculated as usual. The column void time (t_0) was measured by injection of tri-*tert*-butylbenzene as a non-retained sample. Retention times were mean values of two replicate determinations. All separations were carried out at ambient temperature.

3. Results and discussion

3.1. 3,3′-Di(1,3-thiazolidin-4-one) derivatives with two stereogenic centres

The chromatographic results on silica gel for compounds **1–12** are presented in Table 1. Racemate and *meso* compounds were very well separated, exhibiting differences in the k' values of more than 1 and the *meso* form was more retained than the racemic mixture (2*R*,2′*R* and 2*S*,2′*S*) for all the compounds. The retention times were reasonably short and only **11** and **12** required a high content of 2-propanol in hexane for better elution.

The results on Chiralcel OD stationary phase (CSP) are summarized in Table 2. Good separation factors between the enantiomers (α = 1.20–1.66) were obtained, and were slightly improved by a decrease in the percentage of

Table 1
Normal-phase HPLC of compounds **1–16**

Compound	R	R′	Configuration	A (%)[a]	t_1 (min)	k'
1	H	3-Cl	*dl*	5	4.26	0.91
2	H	3-Cl	*meso*	5	7.17	2.22
3	H	3-Br	*dl*	5	4.31	0.94
4	H	3-Br	*meso*	5	7.23	2.24
5	H	3-F	*dl*	10	3.05	0.37
6	H	3-F	*meso*	10	5.19	1.33
7	H	3,4-Cl_2	*dl*	5	5.33	1.39
8	H	3,4-Cl_2	*meso*	5	11.36	4.10
9	H	3-CF_3	*dl*	5	5.91	1.66
10	H	3-CF_3	*meso*	5	8.80	2.95
11	H	3,4-$(OCH_3)_2$	*dl*	5	–[b]	
				30	6.31	1.83
12	H	3,4-$(OCH_3)_2$	*meso*	30	8.59	2.81
13	CH_3	3-F	$(dl)_1(A_1)$[c]	70[d]	6.56[e]	6.29
			$(dl)_2(A_1)$[c]	70[d]	5.90[f]	5.55
14	CH_3	3-F	$dl(A_2)$[c]	70[d]	9.09	9.10
15	CH_3	3-F	dl + *meso*(B_1)[c]	70[d]	15.50[g]	16.22
16	CH_3	3-F	*meso*(B_2)[c]	70[d]	16.43	17.25

[a] Percentage of 2-propanol in *n*-hexane at a flow-rate of 0.3 ml/min, $t_0 = 2.23$ min.
[b] Retained in the column.
[c] See text.
[d] Percentage of dichloromethane in *n*-hexane at a flow-rate of 1.0 ml/min, $t_0 = 0.9$ min.
[e] Elution time of the more abundant diastereomer $(dl)_1$.
[f] Elution time of the less abundant diastereomer $(dl)_2$.
[g] Broad peak with large tail.

2-propanol in the mobile phase. The resolution (R_S) was strongly improved by a decrease in the polarity of the mobile phase and in some instances (**1** and **3**) baseline separation was obtained only by decreasing the polarity of the eluent.

The *meso* forms did not follow a predictable elution order when compared with their *dl* forms, some of them possessing a higher retention factor than those of the enantiomers (**1** and **2**, **3** and **4**, and **5** and **6**), some having intermediate elution times with respect to the enantiomers (**7** and **8**, and **11** and **12**). The R′ substituent in the 2,2′-aryl groups strongly influences the interaction with the CSP and a different polarity of the mobile phase was necessary for reasonable elution of various compounds, as shown by the comparison of the behaviours of **1** and **11**.

Typical chromatograms are shown in Figs. 2 and 3, which show how the enantiomeric resolution was affected by the polarity and the flow-rate of the mobile phase. Elution of the *meso* form as a mixture with the *dl* form is also shown in Fig. 3.

The resolution (R_S) of compound **5** afforded a milligram-scale separation and measurement of the CD spectra of the enantiomeric pair, by repeated 50-μl injections of the racemic compound and collection of the eluate from the two chromatographic peaks. The CD spectra were mirror images of each other, as shown in Fig. 4. Analytical HPLC re-runs of the eluates indicated 100% enantiomeric purity of the first peak and 97% enantiomeric purity of the second peak. Their UV spectra were also identical.

We should mention also that the X-ray structures of the *meso* compound **6** and of the racemic

Table 2
Resolution of compounds **1–12** by HPLC on Chiralcel OD

Compound	R	R′	Configuration	A (%)[a]	k'_1		k'_2	α	R_S
1	H	3-Cl	*dl*	10	3.09		4.04	1.31	3.0
				30	1.36		1.67	1.23	1.0
2	H	3-Cl	*meso*	30		2.13			
3	H	3-Br	*dl*	30	1.88		2.26	1.20	1.4
				70	1.21		1.46	1.20	0.8
4	H	3-Br	*meso*	70		1.77			
5	H	3-F	*dl*	30	1.72		2.22	1.29	1.6
				70	0.79		0.98	1.24	1.1
6	H	3-F	*meso*	70		1.21			
7	H	3,4-Cl_2	*dl*	30	1.74		2.90	1.66	3.6
				70	1.20		1.86	1.55	2.5
8	H	3,4-Cl_2	*meso*	70		1.47			
9	H	3-CF_3	*dl*	30[b]	1.02		1.44	1.41	2.4
				30	1.12		1.56	1.39	1.9
				50[b]	0.68		0.95	1.39	1.7
				50	0.54		0.75	1.38	1.2
10	H	3-CF_3	*meso*	50		0.90			
11	H	3,4-$(OCH_3)_2$	*dl*	70	2.32		3.44	1.48	2.3
				70[b]	3.03		4.57	1.51	2.9
				90	2.83		4.21	1.48	1.9
				90[b]	3.23		4.72	1.46	2.7
12	H	3,4-$(OCH_3)_2$	*meso*	70		2.67			

[a] Percentage of 2-propanol in *n*-hexane at flow-rate of 1.0 ml/min, unless specified otherwise, $t_0 = 3.80$ min.
[b] Flow-rate 0.5 ml/min, $t_0 = 6.45$ min.

5 established their absolute stereochemistry [9], leading to a correction of a previously reported NMR assignment [2].

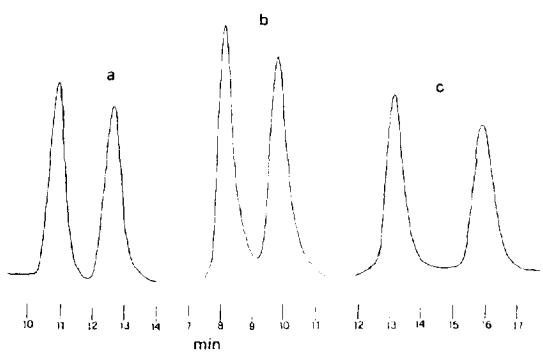


Fig. 2. HPLC separation of the enantiomeric pair of compound **9**. Mobile phase: *n*-hexane–2-propanol. (a) 5:5 at 0.5 ml/min, (b) 7:3 at 1 ml/min, (c) 7:3 at 0.5 ml/min.

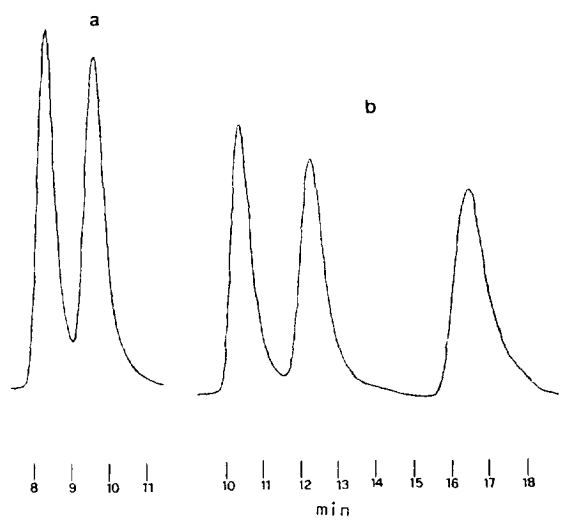


Fig. 3. HPLC separation (mobile phase: *n*-hexane–2-propanol) at 1 ml/min. Mobile phase composition: (a) compound **5**, 5:5; (b) compounds **5** and **6**, 7:3: first and second peaks, *dl* pair; third peak, *meso*.

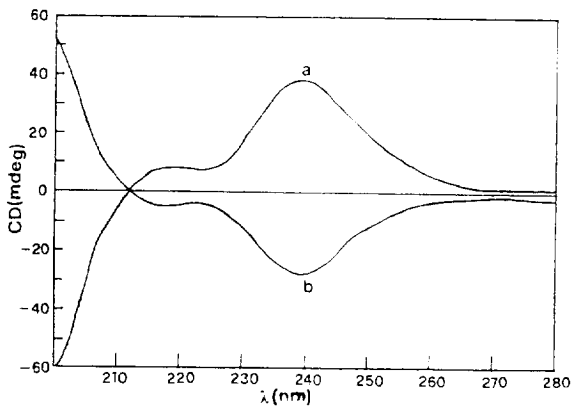
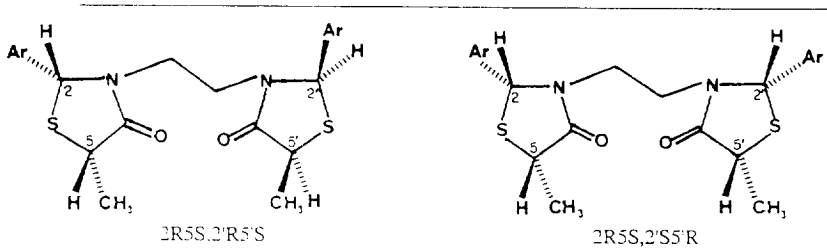


Fig. 4. CD spectra of the enantiomeric pair of compound **5** obtained from the first-eluted peak (positive) and from the second-eluted peak (negative) in ethanol at 25°C.

3.2. 3,3′-Di(1,3-thiazolidin-4-one) derivatives with four stereogenic centres

The chromatographic behaviour of **13–16** was more complicated. In fact, the nucleophilic addition of racemic α-mercaptopropionic acid to the N,N′-dibenzylideneethylenediamine resulted in ten possible stereoisomers with four stereogenic centres, as reported in Fig. 5. A degeneracy due to the presence of *meso* isomers reduced the maximum number of stereoisomers from the expected sixteen to ten. Two of these centres (2 and 2′) bear the same groups, and the remaining two (5 and 5′) also bear the same groups, but these are different from the groups at 2 and 2′. In this situation, four enantiomeric pairs and two *meso* forms exist; all of them were separated and

	C-2	C-2′	C-5	C-5′	2-5/2′-5′	Type	Compd.
Enantiomers	R	R	R	R	trans/trans	A	13
	S	S	S	S		A	
Enantiomers	R	R	R	S	trans/cis	A	13
	S	S	S	R		A	
Enantiomers	R	R	S	S	cis/cis	A	14
	S	S	R	R		A	
Enantiomers	R	S	R	R	trans/cis	B	15
	S	R	S	S		B	
Meso	R	S	R	S	trans/trans	B	15
	R	S	S	R	cis/cis	B	16



2R5S,2′R5′S 2R5S,2′S5′R

Fig. 5. Possible diastereomers of a 3,3′-di(1,3-thiazolidin-4-one) bearing a 3-fluorophenyl group in positions 2 and 2′. Stereochemistries of the enantiomer *2R5S,2′R5′S* of the racemic compound **14** and of the *2R5S,2′S5′R meso* compound **16** are shown. H-2 and H-5 exhibit an NOE effect in ^{1}H NMR spectra in a *cis* relative configuration. Ethylene protons appear as an AA′XX′ system in type A compounds and as an AA′BB′ system in type B compounds.

identified using polysaccharide-derived CSPs, as shown in Fig. 5.

From repeated crystallizations in methanol and radial TLC of the reaction mixture, partial purification resulted in four products. These appeared on silica gel TLC plates [$CHCl_3$–Et_2O (9:1) as the eluent] as two pairs of close spots, A_1 and A_2 at R_F 0.93 and 0.90, respectively, and B_1 and B_2 at R_F 0.74 and 0.67, respectively. In a detailed ^1H NMR study, we found that, in analogues with two stereogenic centres, an *RR* (or *SS*) relative configuration of the 2,2′-carbons causes the appearance of the ethylene protons as an AA′XX′ system (type A ^1H NMR spectra), whereas an *RS* (or *SR*) relative configuration gives an AA′BB′ system (type B ^1H NMR spectra) [2,3]. This observation applies also to type A and type B products, respectively, carrying four stereogenic centres [8], and it is summarized in Fig. 5. In addition, in the ^1H NMR spectra of **14** (A_2) and **16** (B_2), protons 2 and 5 (or 2′ and 5′) reciprocally exhibited a good nuclear Overhauser enhancement (NOE) effect which established a *cis* configuration between them. Also, the 5-methyl irradiation did not give an NOE effect on the proton 2. These results established a racemic 2*R*5*S*,2′*R*5′*S*/2*S*5*R*,2′*S*5′*R* structure for **14** and a *meso* 2*R*5*S*,2′*S*5′*R* structure for **16**, as shown in Fig. 5. Moreover, these results ruled out a "mixed" *trans*/*cis* relationship, i.e., a 2*S*5*S*, 2′*S*5′*R* stereochemistry.

The results of normal-phase and chiral liquid chromatography are summarized in Tables 1 and 3, respectively. On silica gel HPLC, the products B_2 and A_2 were slightly impure (6%) with the diastereomers B_1 and A_1, respectively. Instead, the product B_1 appeared as a broad peak and the width increased with decreasing eluent polarity, suggesting that it is an unseparable mixture of two diastereomers. Co-injection with B_2 gave, however, two distinct peaks, thus ruling out the possibility of B_2 being one of the diastereomers

Table 3
HPLC resolution of the stereoisomers **13**–**16** (R = CH_3, R′ = 3 – F in Fig. 1)

Compound	Configuration	A (%)[a]	k'_1	k'_{meso}	k'_2	α	R_S
13	$(dl)_1$	5	1.47		3.67	2.49	6.3
		10	1.07		2.27	2.13	5.4
	$(dl)_1$	7[b]	3.18		6.77	2.13	9.8
	$(dl)_2$	7[b]	4.89		11.49	2.35	12.5
14	*dl*	5	1.25		1.45	1.15	0.9
		10	0.89		1.02	1.14	0.6
		30	0.46			NS[c]	
15	*dl* + *meso*	7[d]	3.48	3.66	5.81	1.67	3.4
		10[d]	2.75	2.91	4.04	1.47	2.5
		10	2.48	2.85	4.07	1.64	3.5
		15	1.86	2.08	2.40	1.29	1.6
		15[e]	1.45	2.79[f]	2.79		
		25[e]	1.22	1.39[f]	1.39		
		30	0.75	1.02	0.88	1.18	0.5
		30[e]	0.76	1.05	0.91	1.20	0.7
16	*meso*	10		2.94			
		15		2.08			
		30		1.06			

[a] Percentage of 2-propanol in *n*-hexane at a flow-rate of 1 ml/min, unless specified otherwise, t_0 = 3.65 min.
[b] Stationary phase Chiralpak AD, t_0 = 3.91 min.
[c] Not separated.
[d] Flow-rate 1.2 ml/min, t_0 = 2.90 min.
[e] Flow-rate 0.7 ml/min, t_0 = 4.90 min.
[f] *Meso* compound overlapped with the last-eluting enantiomer.

in B_1. In addition, from Table 1, it can be observed that the pair A_1–A_2 had an elution time range much lower than the pair B_1–B_2, in agreement with the TLC results.

Confirmation of the *meso* nature of the stereoisomer **16** came from the chromatographic behaviour on Chiralcel OD. Compound **16** appeared as a unique, sharp peak, as shown in Table 3, with various percentages of 2-propanol in hexane.

In Chiralcel OD chromatography **14** appeared as two distinct peaks of the same area, as shown in Table 3 and Fig. 6. Therefore, it is a racemic compound that, according to the ^{1}H NMR features illustrated above, is identified as the enantiomeric pair *2R5S,2'R5'S/2S5R,2'S5'R* (Fig. 5). It is interesting to note the crucial effect of the polarity of the mobile phase on the enantiomeric resolution of **14**. A concentration of 30% of 2-propanol in hexane did not afford resolution and resulted in a single peak on Chiralcel OD, as shown in Fig. 6c.

In Chiralced OD chromatography **13** (A_1) appeared as two distinct major peaks of the same area at $k' = 1.47$ and 3.67 on eluting with *n*-hexane–2-propanol (95:5), as shown in Table 3, accompanied by a minor distinct peak ($k' = 2.58$, same eluent). This behaviour was maintained at various percentages of 2-propanol in *n*-hexane and various detection wavelengths.

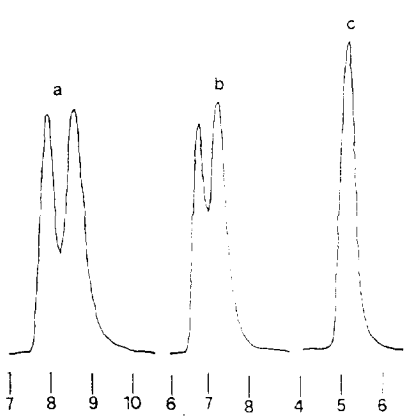


Fig. 6. HPLC separation of the enantiomeric pair of compound **14**. Mobile phase: *n*-hexane–2-propanol at 1 ml/min: (a) 95:5; (b) 9:1; (c) 7:3.

However, as one *meso* form was identified as **16**, as shown above, and the only other *meso* form possible according to Fig. 5 was identified as **15** (as shown below), the appearance of a single minor peak was suspicious. Therefore, we chromatographed **13** on a chiral stationary phase, Chiralpak AD, structurally similar to Chiralcel OD (the same carbamate moieties) but with a wider helicity deriving from the amylose matrix. In this experiment, as shown in Table 3 and Fig. 7, **13** was resolved into two enantiomeric pairs, $(dl)_1$ more abundant and $(dl)_2$ much less abundant. The relative areas of the peaks were confirmed in several experiments. Moreover, the area ratios of the diastereomers in Fig. 7a and b are approximately the same, as expected. Thus, according to the A nature of the ^{1}H NMR spectrum of **13**, as shown in Fig. 5, the two racemates resolved were *2R5R,2'R5'S/2S5S,2'S5'R* and *2R5R,2'R5'R/2S5S,2'S5'S*. The resolution for both enantiomeric pairs was excel-

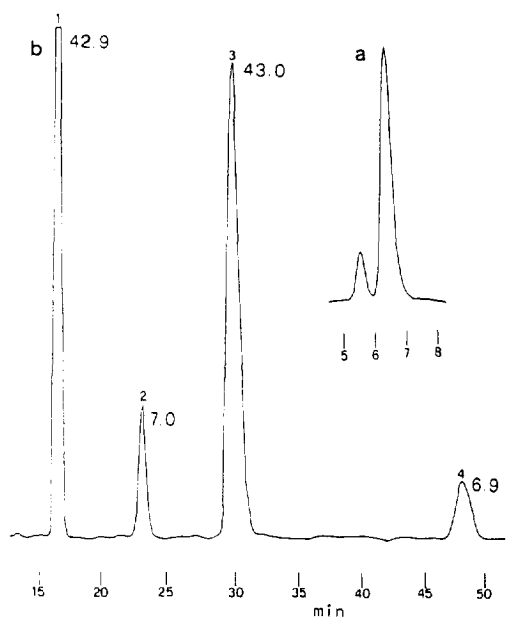


Fig. 7. HPLC separation of the enantiomeric pairs in product **13** (a) on silica gel, mobile phase *n*-hexane–dichloromethane (3:7) at 1 ml/min; (b) on Chiralpak AD, mobile phase *n*-hexane–2-propanol (93:7) at 1 ml/min. Numbers near the peaks represent the relative areas. Peaks 1 and 3 represent the separation of $(dl)_1$; peaks 2 and 4 represent the separation of $(dl)_2$.

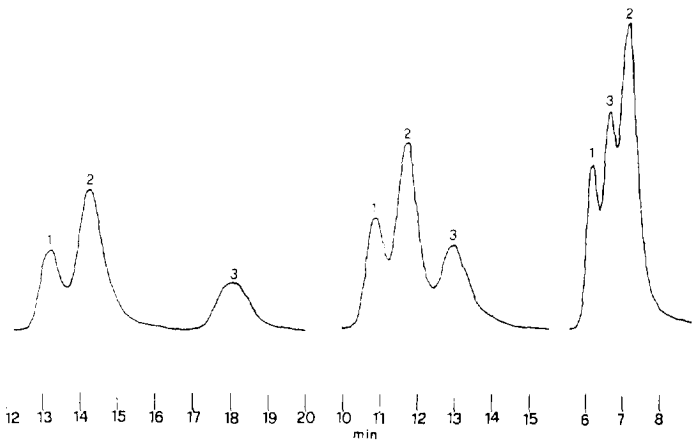


Fig. 8. HPLC separation of the enantiomeric pair and *meso* form in product **15**. Mobile phase: *n*-hexane–2-propanol at 1 ml/min: (a) 9:1; (b) 85:15; (c) 7:3. Peaks 1 and 3 represent the separation of dl; peak 2 is due to the *meso* form.

lent ($R_S = 9.8$ and 12.5), and this can afford a milligram-scale separation of the enantiomers for pharmacological assays. However, k'_2 of $(dl)_2$ was extremely high (11.49, corresponding to an elution time of 48.8 min).

The chromatographic behaviour on Chiralcel OD of **15** (B_1) was also complex. In fact, as shown in Table 3 and Fig. 8, this compound exhibited three peaks under several experimental conditions (various polarities and flow-rates of the mobile phase). Peaks 1 and 3 can be attributed to an enantiomeric pair as their area ratio is about 1 and it remains constant at various polarities and flow-rates of the mobile phase. Their separation and resolution are good and also in this instance a semi-preparative isolation of pure enantiomers appears feasible. The peak 2 can instead be attributed to the remaining *meso* structure (Fig. 5). In fact, this peak did not split under the various experimental conditions reported in Table 3 and also under other conditions not reported. Moreover, the ^{1}H NMR spectrum of **15** suggests clearly an *RS* configuration at the 2,2′ centres (AA′BB′ ethylenic system). This steric requirement is present only in the remaining *meso* form 2*R*5*R*,2′*S*5′*S* and in the racemic 2*R*5*R*,2′*S*5′*R*/2*S*5*S*,2′*R*5′*S* structure, thus identifying also peaks 1 and 3.

In conclusion, by chiral HPLC we separated all ten stereoisomers from the reaction of *dl*-thiolactic acid with N,N′-dibenzylideneethylenediamine. For **13** the excellent resolution opens the way to the isolation of its enantiomers in sizeable amounts. Analogously, by choosing the optimum eluent composition, the last-eluting enantiomer present in **15** can be isolated in pure form and the first-eluting enantiomer can also be isolated, although probably impure with the *meso* diastereomer (peak 2). The stereochemistry and the pharmacological activities of these individual stereoisomers will be investigated.

References

[1] M.G. Vigorita, T. Previtera, M. Basile, G. Fenech, R. Costa De Pasquale, F. Occhiuto and C. Circosta, *Farmaco, Ed. Sci.*, 39 (1984) 1008.

[2] T. Previtera, M. Basile, M.G. Vigorita, G. Fenech, F. Occhiuto, C. Circosta and R. Costa De Pasquale, *Eur. J. Med. Chem.*, 22 (1987) 67.

[3] T. Previtera, M.G. Vigorita, M. Basile, F. Orsini, F. Benetollo, G. Bombieri, *Eur. J. Med. Chem.*, 29 (1994) 317.

[4] A.F. Casy, in D.F. Smith (Editor), *Handbook of Stereoisomers: Therapeutic Drugs*, CRC Press, Boca Raton, FL, 1989, p. 149.

[5] D.G. Cooper, R.C. Young, G.J. Durant and C.R. Ganellin, in C. Hansch (Editor), *Comprehensive Medicinal Chemistry*, Vol. 3, Pergamon Press, Oxford, 1990, p. 371.

[6] *FDA's Policy Statement for the Development of New Stereoisomeric Drugs*. Food and Drug Administration, Washington, DC, 1992.
[7] I. Grillone, *Doctoral Thesis*, University of Messina, Messina, 1992.
[8] M.G. Vigorita, T. Previtera and R. Ottanà, in preparation.
[9] F. Benetollo, G. Bombieri, A. Del Pra, M. Basile, T. Previtera and M.G. Vigorita, *J. Crystallogr. Spectrosc. Res.*, 21 (1991) 113.