ENANTIOMERIC RESOLUTION OF 3,5'-DIMETHYL-4,4'-DIBROMO-1,1'-BISPYRAZOLYL-PHENYLMETHANE BY LIQUID CHROMATOGRAPHY ON TRIACETYLCELLULOSE

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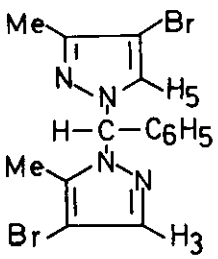
Abstract -The experimental conditions to determine the optical purity of the title compound 1 have been found studying the effect of lanthanide shift reagents (LSR), Eu(fod)$_3$ and Eu(tfc)$_3$. The obtention of enriched enantiomers (57.5%) in (+)-1 and 62% in (-)-1) was possible by the use of liquid chromatography over triacetylcellulose. The compound was devoid of any significant antifungal activity.

3,5'-Dimethyl-4,4'-dibromo-1,1'-bispyrazolylphenylmethane 1 is structurally related to the important antifungal agent clotrimazole[1] 2. In order to test the fungicide activity of racemic 1 and its two enantiomers, we decided to attempt the separation of (+) and (-)-1. There are no reported data about optically active arylheteroarylmethanes and, since liquid chromatography on triacetylcellulose had successfully been applied to other chiral aromatic compounds,[2,3] we used this method for separation.

Enantiomeric distinction and enrichment determinations were achieved by ^{1}H nmr using chiral tris[3-(2,2,2-trifluoro-1-hydroxyethylidene)-d-camphorato]europium (III), Eu(tfc)$_3$. Initially we studied the behaviour of (±)-1 in the presence of tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanodionato) europium (III), Eu(fod)$_3$, and the results were compared with those of the same LSR on 3,3'-dimethyl-4,4'-dibromo-1,1'-bispyrazolylphenylmethane 3, achiral isomer of 1. The synthesis and structural characteristics of 1 and 3 had been recently reported.[4]



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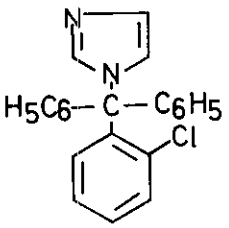

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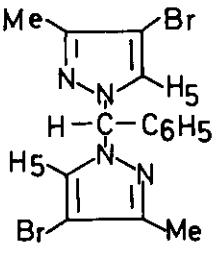

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For both compounds, 1 and 3, the largest induced chemical shifts in ^{1}H nmr ($CDCl_3$ at 300 MHz) produced by addition of Eu(fod)$_3$ were found for the methine protons (H-C$_1$) as it can be seen in

Tables 1 and 2. The addition of a small amount of $Eu(fod)_3$ on 1 (molar ratio 0.113), affected the H_5 signal more than the one corresponding to the HC_1 group, suggesting that a conformation of type A (with opposite oriented pyrazoles) would be predominant. The LSR would approach the N lone pairs, first as in A_1 and later as in A_2. Crossing plots are well documented for multidentate ligands.[6,7]

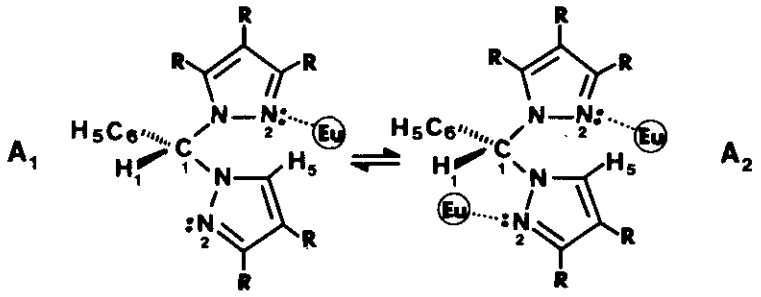

TABLE 1.-Chemical shifts δ in Hz at 300 MHz and $\Delta\delta$ in Hz; intercept (Y_0); slope (m) and squared regression coefficients (R^2) for protons of compound 1 with $Eu(fod)_3$

Molar ratio $Eu(fod)_3$/1	H_3		H_5		HC_1		CH_3-3		CH_3-5	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
0	2246.3	0	2257.7	0	2264.5	0	676.4	0	710.2	0
0.113	2253.1	6.8	2274.3	16.6	2274.3	9.8	682.0	5.6	715.9	5.7
0.249	2276.5	30.2	2299.7	42.0	2344.6	80.1	704.6	28.2	735.2	25.0
0.438	2313.7	67.4	2343.0	85.3	2455.1	190.6	741.6	65.2	765.9	55.7
0.887	2378.4	132.1	2419.2	161.5	2646.8	382.3	806.3	129.9	819.0	108.8
Y_0	-5.1		-1.6		-20.7		-5.9		-4.1	
m	155.2 (34)		185.7 (41)		454.2 (100)		153.3 (34)		127.8 (28)	
R^2	0.992		0.997		0.989		0.991		0.992	

TABLE 2.-Chemical shifts δ in Hz at 300 MHz and $\Delta\delta$ in Hz; intercept (Y_0); slope (m) and squared regression coefficients (R^2) for protons of compound 3 with $Eu(fod)_3$

Molar ratio $Eu(fod)_3$/3	H_3		HC_1		CH_3-3	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
0	2216.1	0	2232.4	0	676.5	0
0.174	2241.5	25.4	2335.3	102.9	687.6	11.1
0.352	2255.5	39.4	2389.9	157.5	693.5	17.0
0.617	2283.7	67.6	2504.1	271.6	707.2	30.7
1.383[a]	2328.9	112.8	2674.6	442.2	724.4	47.9
Y_0	2.7		11.1		0.9	
m	106.3 (25)		426.6 (100)		48.3 (11)	
R^2	0.989		0.988		0.990	

[a] This value has not been included in the regression because the nonlinear region has been reached.[8]

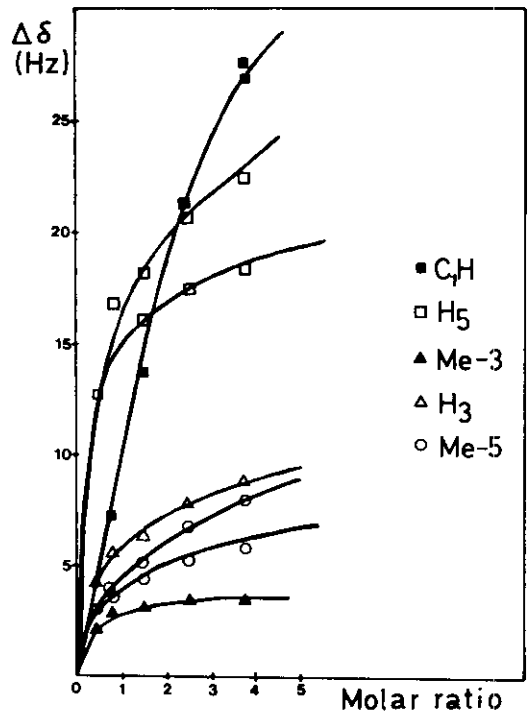

Fig. 1. Dependence of proton induced shifts of (±)-1 with molar ratio Eu(tfc)$_3$/1

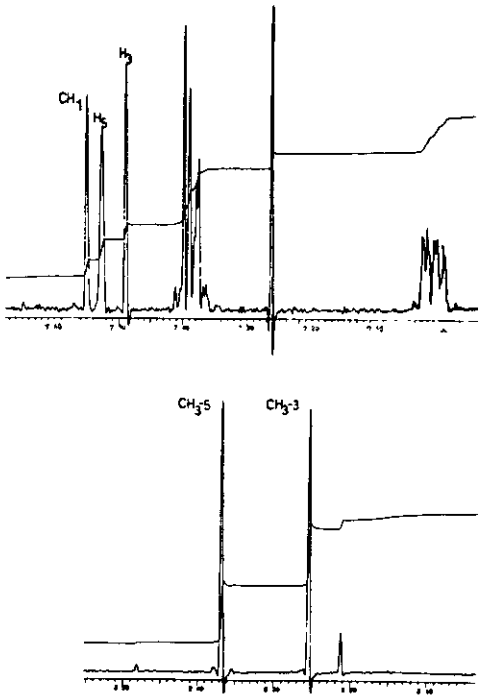

Fig. 2a. ^{1}H nmr spectrum of (±)-1 in $CDCl_3$ at 300 MHz

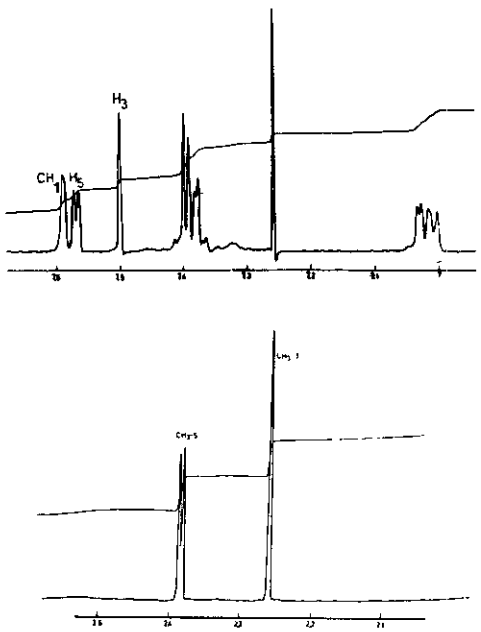

Fig. 2b. ^{1}H nmr spectrum of (±)-1 in the presence of Eu(tfc)$_3$. $CDCl_3$ at 300 MHz. Molar ratio: 1.428

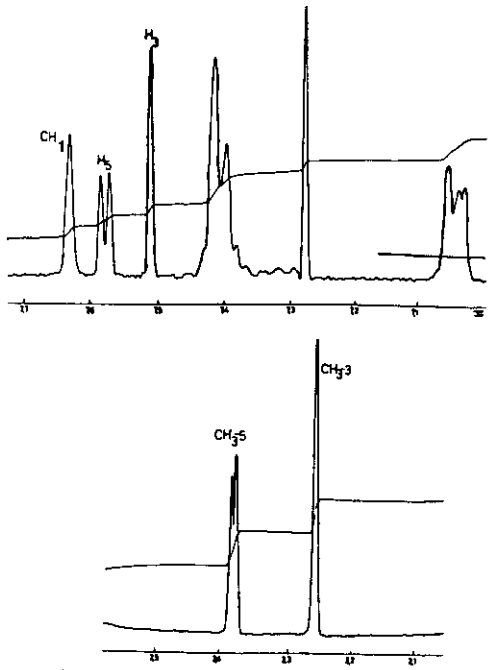

Fig. 2c. ^{1}H nmr spectrum of (±)-1 in the presence of Eu(tfc)$_3$. $CDCl_3$ at 300 MHz. Molar ratio: 3.687

No difficulties were found in the assignment of ^{1}H nmr signals to the different protons, except for H_5 and HC_1 in compound $\underset{\sim}{1}$. The assignment of the signal at 2646.8 Hz (Table 1, molar ratio: 0.887) to the HC proton was made by selective decoupling of the methine carbon signal in the ^{13}C nmr spectrum.

The results obtained with compound $\underset{\sim}{1}$ and $Eu(tfc)_3$ in $CDCl_3$ at 300 MHz are gathered in Table 3. Due to the weak Lewis acid character[9] of this LSR it was necessary to use larger molar ratios $Eu(tfc)_3/\underset{\sim}{1}$. Lanthanide-induced shifts (Hz) variation with increasing amounts of LSR, molar ratio $Eu(tfc)_3/\underset{\sim}{1}$, can be represented by two straight lines intersecting at approximately a molar ratio = 1 (Fig. 1).

Chemical shift non equivalences of enantiomers appear for H_5 and CH_3-5 protons at molar ratio of 1.428 and for the methine at molar ratio of 3.687. The other signals do not show any splitting in the experimental conditions used in this study. Further examples of chiral nmr recognition by optically active LSR in which fairly well shifted signals are not splitted while less shifted ones split, are known in the literature.[10] In our case, the best conditions for the determination of optical purity are obtained when $\Delta\delta(H_5)$ = 4 Hz, $\Delta\delta(CH_3\text{-}5)$ = 2.2 Hz and $\Delta\delta$(HC) = 0.4 Hz, which correspond to a $Eu(tfc)_3/\underset{\sim}{1}$ molar ratio of about 3.5 (See Fig. 2).

TABLE 3.-Chemical shifts δ in Hz at 300 MHz and $\Delta\delta$ in Hz; intercept (Y_0); slope (m) and squared regression coefficients (R^2) for protons of compound (±)-$\underset{\sim}{1}$ with $Eu(tfc)_3$

Molar ratio $Eu(tfc)_3/\underset{\sim}{1}$	H_3		H_5		HC_1		CH_3-3		CH_3-5	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
0	2243.1	0	2252.4	0	2261.9	0	674.8	0	708.8	0
0.361	2247.3	4.2	2264.9	12.5	2266.0	4.1	676.8	2.0	712.0	3.2
0.737	2248.6	5.5	2269.1	16.7	2269.1	7.2	677.6	2.8	712.7	3.9
Y_0	0.5		1.5		0.2		0.2		0.4	
m	7.5 (33)		22.6 (100)		9.7 (43)		3.8 (17)		5.3 (23)	
R^2	0.917		0.917		0.992		0.924		0.878	
1.428	2249.3	6.2	2270.4	18.0(+)	2275.5	13.6	677.8	3.0	713.8	5.0(+)
			2268.4	16.0(-)					713.0	4.2(-)
2.458	2250.8	7.7	2273.1	20.7(+)	2283.0	21.1	678.2	3.4	715.6	6.8(+)
			2269.8	17.4(-)					714.0	5.2(-)
3.687	2251.7	8.6	2274.8	22.4(+)	2289.1	27.2(-)	678.1	3.3	716.7	7.9(+)
			2270.8	18.4(-)	2288.7	26.8(-)			714.5	5.7(-)
Y_0	4.9		15.5(+)		5.5(+)		2.8		3.4(+)	
			14.6(-)		5.8(-)				3.4(-)	
m	1.1 (18)		1.9(+) (32)		6.0(+) (100)		0.2 (2.6)		1.3(+) (21)	
			1.1(-) (18)		5.8(-) (97)				0.6(-) (11)	
R^2	0.956		0.960(+)		0.987(+)		0.617		0.959(+)	
			0.970(-)		0.983(-)				0.962(-)	

The racemic 3,5'-dimethyl-4,4'-dibromo-1,1'-bispyrazolylphenylmethane 1 was resolved into its two enantiomers by liquid chromatography on microcrystalline triacetylcellulose (See Fig. 3 and experimental part). Enantiomeric enrichments determined by ^{1}H nmr with Eu(tfc)$_3$ were 62% in (-)-1 and 57.5% in (+)-1, corresponding to an absolute $[\alpha]_D^{30} = 50.5°$. These experiments allow simultaneously to assign high field CH_3-5 and H_5 signals to the (-)-enantiomer.

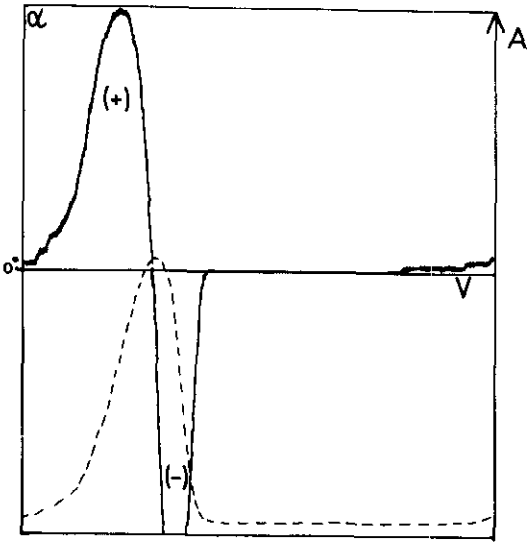

Fig. 3. Chromatogram of 100 mg of (±)-1 in 5 ml of 95 ethanol after three cycles through two columns of triacetylcellulose (particle size 15-25 μ) (equivalent length of analysis: 1.20 meter). α : Rotation angle (-) at 436 nm. A: Absorbance (----) at 254 nm; V: volume of eluate.

To study the possibility of racemization of the enantiomers we carry out the following experiment: an equimolar mixture of (+)-1 and trityl chloride in methylene chloride was prepared. This solution is stable at room temperature, after 50 mn the rotatory power remains unchanged. After 24 h at reflux, the solution does not show any rotatory power. However, a 300 MHz ^{1}H nmr spectrum presents the signals corresponding to a complex mixture where those of compounds 1 and 3 were clearly identified. The most reasonable interpretation of this result is a breaking of the N-C bond between 4-bromo-5-methyl-pyrazolyl residue and the methine carbon, followed by a recombination yielding both isomers.

Compounds 1 and 3 did not show any significant activity against *B. cinera, A. flavus, M. rouxii* and *C. albicans* at doses as high as 640 μg/ml. Clotrimazole against the same fungi was active at approximately 1 μg/ml.

EXPERIMENTAL

^{1}H nmr spectra were recorded on Varian XL-300 superconducting spectrometer. Chemical shifts in Hz were measured in $CDCl_3$ referred to TMS as an internal standard. Compounds 1 and 3 are described in the original paper.[4] Eu(fod)$_3$ and Eu(tfc)$_3$ were purchased from E. Merck, Darmstadt. Weighed amounts of LSR were added in increments to achieve different lanthanide/substrate molar ratios and the corresponding lanthanide induced chemical shifts were carefully measured.

Separation by Liquid Chromatography on Triacetylcellulose (TAC)

The technique and apparatus for separation of enantiomers on TAC have been described by several authors.[2,11,12]

Analytical: A solution of 5 mg of racemic 1 in 2 ml of ethanol 95% is injected in one column A (length, 20 cm; internal diameter, 2.5 cm; phase, TAC 15-25 microns; flow rate: 138 ml/h; pressure drop: 1.35 bar; temperature, 25°C and a k=0.53 was determined using 1,3,5-tri-tert-butylbenzene as reference.[2] A LKB 2138 UVICORD S detector (λ = 254 nm) and a 241 MC Perkin Elmer polarimeter (λ = 436 nm) were used for the detection of the compounds. Even if that was not possible to separate the two enantiomers, (α close to 1), the presence by polarimetric means of a (+) and a (-) signal (Actual volume of eluate = 100 ml) pressed us to use a recycling technique on TAC to get the desired enantiomeric enrichment.

Semi-preparative: One hundred mg of (±)-1 dissolved into 5 ml of ethanol 95% were injected in two A columns in series at a flow rate of 115 ml/h. After three cyclic passages of the central fraction, eluted at retention volumes of eluate comprised between 180-222 ml for the first run to eliminate small impurities at the front and at the tail of the peak, 25 mg of (-)-enantiomer as a first fraction [62% (-) and 38% (+) according to ^{1}H nmr with Eu(tfc)$_3$] and 38 mg of (+)-enantiomer as a second fraction [57.5% (+) and 42.5 (-)] were obtained.

ACKNOWLEDGEMENTS

This work has been financed by CAICYT of Spain (Project Number 326/84). We are indebted to Dr. G. Gomez Alarcon of "Centro de Investigaciones Biologicas" (CSIC, Madrid) who kindly run the biological assays.

REFERENCES

1. L. Zirngibl, Progress in Drug Research, 1983, 27, 253.
2. A. Mannschreck, H. Koller, and R. Wernicke, Kontakte, 1985, 1, 40.
3. J. Elguero, R.M. Claramunt, Y. Shindo, M. Mukai, C. Roussel, A. Chemlal, and A. Djafri, Chem. Scripta, 1987, 27, 283.
4. P. Ballesteros, J. Elguero, and R.M. Claramunt, Tetrahedron, 1985, 41, 5955.
5. R.M. Claramunt, H. Hernandez, J. Elguero, and S. Julia, Bull. Soc. Chim. France, II, 1983, 5.
6. O. Hofer, in 'Topic in Stereochemistry' (N.L. Allinger and E.L. Eliel, Eds.), John Wiley and Sons, New York, 1976, Vol. 9, p. 142.
7. L. La Planche, Org. Magn. Reson., 1980, 14, 40.
8. J.W. ApSimon, H. Beierbeck, and A. Fruchier, Can. J. Chem., 1972, 50, 2725.
9. G.M. Whitesides and D.W. Lewis, J. Am. Chem. Soc., 1971, 93, 5914.
10. W. Offermann and A. Mannschreck, Org. Magn. Reson., 1984, 22, 355 and references therein.
11. A. Eiglsperger, F. Kastner, and A. Mannschreck, J. Mol. Struct., 1985, 126, 421.
12. R. Isaksson and J. Rochester, J. Org. Chem., 1985, 50, 2519.

Received, 29th May, 1987