Journal of Chromatography, 355 (1986) 354–357
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 325

Note

Enantiomeric separation of D,L-Dns-amino acids by one- and two-dimensional thin-layer chromatography

ROSANGELA MARCHELLI*, ROBERTA VIRGILI, ELISABETTA ARMANI and ARNALDO DOSSENA
Istituto di Chimica Organica dell'Universita', Via M. D'Azeglio 85, I-43100 Parma (Italy)
(Received November 6th, 1985)

The chiral resolution of amino acids is becoming increasingly important in organic synthesis, drug design, biology and medicine. Although very efficient gas[1–3] and liquid[4–7] chromatographic methods have become available in the recent years, a simple and rapid method of monitoring optical purity is still desirable.

Only recently was the first enantiomeric separation of Dns-amino acids by thin-layer chromatography (TLC) reported by Weinstein[8], who used reversed-phase plates, pre-treated with a copper(II) complex of N,N-di-*n*-propyl-L-alanine and eluted with an eluent preferably containing the chiral complex. The method was further developed by Grinberg and Weinstein[9], who devised a two-dimensional technique, involving an elution gradient in the first dimension, subsequent treatment of the plates with the chiral complex and a temperature gradient in the second dimension.

In a systematic project aiming at studying the mechanism of chiral recognition, we have recently reported[10] the resolution of D,L-Dns-amino acids by reversed-phase high-performance liquid chromatography (HPLC) using copper(II) complexes of chiral ligands of the type AA-NN-*n* reported in Fig. 1. In these ligands two L-amino acids are joined via an amide bond by ethylene and trimethylene bridges and are endowed with varying degrees of lipophilicity and bulkiness, depending on the nature of the amino acid side chain (AA = alanine, valine, phenylalanine).

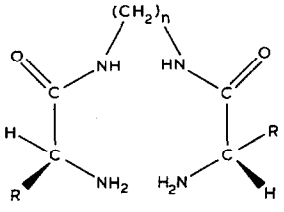


Fig. 1. Ligands AA-NN-n: n = 2, 3; R = $C_6H_5CH_2$ (Phe), $(CH_3)_2CH$ (Val), CH_3 (Ala).

We report here that by impregnating HPTLC RP-18 plates with these ligands and copper acetate at various pH, we were able to resolve D,L-Dns-amino acids in both one-dimensional and two-dimensional arrangements with or without a chiral additive in the eluent (water–acetonitrile) and under isocratic conditions.

0021-9673/86/$03.50

EXPERIMENTAL

The Dns-amino acids were purchased from Sigma (St. Louis, MO, U.S.A.).

Ligands AA-NN-2 and AA-NN-3 were synthesized[11] as dihydrochlorides by condensation of the (*Z*)-N-amino acid hydroxysuccinimide esters with 1,2-ethane- or 1,3-propanediamine under basic conditions (triethylamine) and successive deprotection by hydrogenolysis and treatment with methanol–hydrochloric acid.

One-dimensional separation

RP-18 $F_{254}S$ HPTLC plates (Merck), 10 × 20 cm, for nanochromatography were immersed for 1 h in a 0.3 *M* solution of sodium acetate in water–acetonitrile (60:40), adjusted to pH 7.5 with acetic acid. In order to obtain a uniform deposition of sodium acetate on the plates, immersion is preferred to elution.

After drying at 100°C in an oven for 1 h and cooling to room temperature, the plates were immersed for at least 2 h in water–acetonitrile (10:90) containing the ligand Phe-NN-2 (4 m*M*) and copper acetate (4 m*M*). Experiments were performed in the pH range 6.8–8.0. The plates were dried at 60°C for 1 h and were ready for use or stored for later use.

Dns-amino acids in aqueous solution were applied to the plates with a microsyringe and developed in water–acetonitrile at different pHs, the percentage of acetonitrile and the pH depending on the type of the amino acid to be separated. The conditions are reported in Table I. The Dns-amino acids were detected with a fluorescent lamp (365 nm). The elution order was always verified by comparison with an authentic sample of the L-enantiomer.

Two-dimensional separation

Stratocrom SIF_{254} $C_{18}W$ reversed-phase TLC plates (Carlo Erba), 10 × 10 cm (thickness 0.20 mm), were pre-developed with methanol, dried for 30 min at 100°C, immersed in the same buffer solution as described for one-dimensional separation, dried at 100°C and cooled to room temperature. Then they were immersed vertically in water–acetonitrile (10:90) containing the ligand Phe-NN-2 (4 m*M*) and copper acetate (4 m*M*) at pH 7 for 4 h, leaving a 2-cm wide strip out of the solution and covered with a glass plate. The plate was then dried at 60°C for 1 h.

First development. After application of the mixture of D,L-Dns-amino acids at one corner of the plate not impregnated with the complex, the plate was developed along the untreated strip with a solution of 0.25 *M* sodium acetate in water–acetonitrile (70:30) at pH 7. It was proved experimentally that the addition of sodium acetate is necessary for this type of plate because it prevents peeling of the adsorbent layer from the glass.

Second development. After drying, the plate was developed in the perpendicular direction with a solution of 0.3 *M* sodium acetate in water–acetonitrile (60:40) at pH 7.5, thus achieving the separation of the enantiomers.

RESULTS AND DISCUSSION

As far as the copper complexes involved in the separation are concerned, we have verified by potentiometric titration[11] and by UV–VIS and EPR spectroscopy[12]

TABLE I

ENANTIOMERIC RESOLUTION OF D,L-DNS-AMINO ACIDS BY PHE-NN-2–COPPER ACETATE USING HPTLC

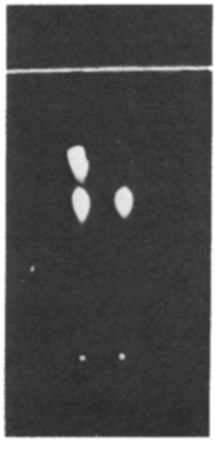
10 × 20 cm plates were used, unless stated otherwise.

Dns-AA	R_F (L)	R_F (D)	$\alpha = R_F$ (D)/R_F (L)	*Eluent*	
				Acetonitrile (%)	*pH*
Glu*,**	0.24	0.40	1.67	33	6.8
Asp*,**	0.09	0.21	2.33	33	6.8
Ser***	0.41	0.51	1.24	50	7.5
Thr***	0.47	0.52	1.11	50	7.5
Met***	0.34	0.40	1.18	50	7.5
Norval***	0.42	0.36	0.86	50	7.5
Leu**	0.47	0.38	0.81	50	7.5
Norleu**	0.42	0.33	0.78	50	7.5
Phe**	0.35	0.41	1.17	50	7.5

* 10 × 10 cm plates.
** Without the complex in the eluent.
*** With the complex (2 m*M*) in the eluent.

that in the pH range 6.8–8 considered here, AA-NN-3 ligands form only one complex with copper acetate, $CuLH_{-2}$, whereas AA-NN-2 give rise to two complexes, $(Cu_2L_2H_{-2})^{2+}$ and $CuLH_{-2}$. The best separation values (α) were obtained with the ligand Phe-NN-2, which resolved most amino acids, as reported in Table I and Fig. 2.

The lipophilicity of the ligand turned out to be of great importance. In fact,



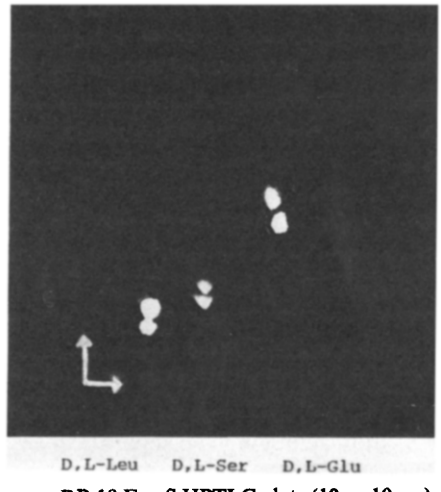


Fig. 2. Enantiomeric separation of D,L-Dns-glutamic acid on an RP-18 $F_{254}S$ HPTLC plate (10 × 10 cm). Eluent: water–acetonitrile (60:40), pH 6.8.

Fig. 3. Two-dimensional enantiomeric separation of Dns-amino acids by HPTLC. First direction, water–acetonitrile (70:30), pH 7; second direction, water–acetonitrile (58:42), pH 7.5, after treatment with the Cu(II)–Phe-NN-2 complex (4 m*M*).

Val-NN-2 gave fairly good results, whereas ligands containing L-alanine were not retained sufficiently on the plate and were mostly eluted along with the solvent front. Ligands of the type AA-NN-3 gave poor or no resolution, in agreement with HPLC results[10].

Surprisingly, whereas all the amino acids tried showed a higher R_F value for the D-enantiomer, leucine, norleucine and norvaline gave a reversed elution order. An example of a chiral separation using the two-dimensional arrangement is illustrated in Fig. 3. Although mixtures of only three or four amino acids have been resolved so far, we are confident that the method can be improved further. The proposed method for the resolution of Dns-amino acids offers all the advantages of TLC and does not require more sophisticated gradient techniques.

ACKNOWLEDGEMENTS

We thank Prof. E. Gil-Av and Dr. S. Weinstein for fruitful discussions. This work was supported by an Italian–Israeli C.N.R. Bilateral Project.

REFERENCES

1 E. Gil-Av, B. Feibush and R. Charles-Sigler, *Tetrahedron Lett.*, (1966) 1009.
2 W.- A. König, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 588.
3 V. F. Shurig, *Angew. Chem., Int. Ed. Engl.*, 23 (1984) 747.
4 V. A. Davankov and Yu. A. Zolotarev, *J. Chromatogr.*, 155 (1978) 285, 295 and 303.
5 E. Gil-Av, A. Tishbee and P. E. Hare, *J. Am. Chem. Soc.*, 102 (1980) 5115.
6 S. Weinstein, *Angew. Chem., Int. Ed. Engl.*, 21 (1982) 218; *Angew. Chem. Suppl.*, (1982) 425.
7 V. A. Davankov, A. A. Kurganov and A. S. Bochkov, *Adv. Chromatogr.*, 22 (1983) 71.
8 S. Weinstein, *Tetrahedron Lett.*, (1984) 985.
9 N. Grinberg and S. Weinstein, *J. Chromatogr.*, 303 (1984) 251.
10 R. Marchelli, A. Dossena, G. Casnati, F. Dallavalle and S. Weinstein, *Angew. Chem., Int. Ed. Engl.*, 24 (1985) 336.
11 E. Armani, G. Casnati, F. Dallavalle, A. Dossena and R. Marchelli, in preparation.
12 E. Armani, C. Giori, R. Marchelli and A. Dossena, in preparation.