Journal of Chromatography, 234 (1982) 485–488
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,313

Note

Improved enantiomeric resolution of D,L-Dns-amino acids

STANLEY K. LAM
Department of Laboratory Medicine, Albert Einstein College of Medicine, Bronx, NY 10461 (U.S.A.)
(Received August 24th, 1981)

Earlier, we reported[1,2] the resolution of optical isomers of D- and L-Dns-amino acids by mixed complex formation with L-proline in the mobile phase[1,2]. Eight pairs of amino acids were separated. Amino acids with small and/or polar alkyl substituents were not resolved. In this note, the improved resolution of Dns-amino acids with Cu(II)–L-proline mobile phase is described. Several of the small polar amino acids were base line resolved. An efficient column and the proper composition of mobile phase are critical in achieving the separation.

The effect of two different column materials on separation were investigated. The packing materials did not affect the stereoselectivity between the D- and L-isomers, but the retention and selectivity between the amino acids were changed. The Cu(II)–proline system is highly selective. We demonstrated that this system is selective not only for the separation of optical isomers of amino acids but for the individual amino acids as well.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a Model Series 2 liquid chromatographic pump, a Model LC 650-10 fluorescence spectrophotometer and a Model 56 chart recorder (Perkin-Elmer, Norwalk, CT, U.S.A.). The analytical columns (15.0 × 0.42 cm) were packed with either LiChrosorb® RP-8 or Spherisorb® C_{18} by the downward slurry technique. Sample was introduced via a Rheodyne 7105 injection valve. The fluorescence at 480 nm was monitored with excitation at 340 nm.

Reagents

Acetonitrile distilled in glass was bought from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). D- and L-Dns-amino acids were bought from Sigma (St. Louis, MO, U.S.A.) and Pierce (Rockford, IL, U.S.A.). Some of the Dns-amino acids were prepared as previously described[1]. The mobile phases were made up of 5 m*M* L-proline, 2.5 m*M* $CuSO^4 \cdot 5H_2O$ and 0.5 g ammonium acetate per liter of deionized water with appropriate percentage of acetonitrile as shown in Figs. 1–3.

0021-9673/82/0000–0000/$02.75

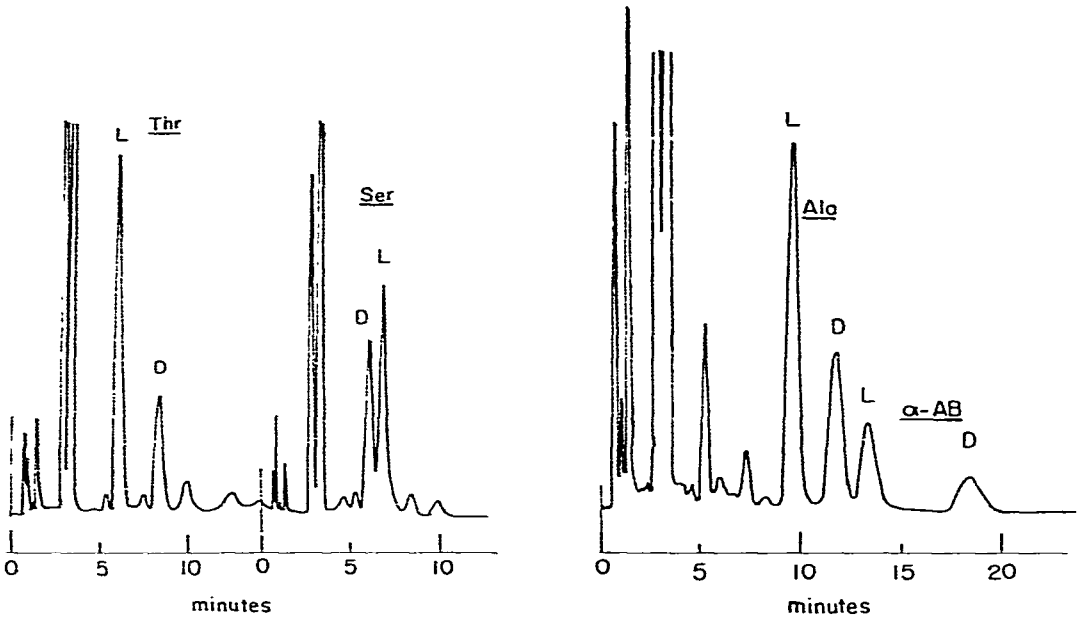

Fig. 1. Separation of D,L-Dns threonine and D,L-Dns serine. Mobile phase: 15% acetonitrile in an aqueous solution containing 5 m*M* L-proline, 2.5 m*M* $CuSO_4 \cdot H_2O$ and 0.5 g ammonium acetate l of deionized water. pH 7.0. Column: 15 × 0.42 cm LiChrosorb RP 8. Flow-rate: 2.0 ml/min.

Fig. 2. Separation of D,L-Dns alanine and D,L-Dns α-amino butyric acid. Conditions as in Fig. 1.

RESULTS AND DISCUSSION

Many D- and L-Dns-amino acids can be separated by a copper (II)–L-proline complex mobile phase. Previously, we achieved the complete resolution of 8 pairs of Dns-amino acids which possess non-polar side chains. The selectivity between the D- and L-pairs is dependent on the alkyl substituent on the α-carbon of the amino acid. The greater the carbon content and the bulkier the alkyl group, the larger is the stereoselectivity because of the interaction of the alkyl groups of the bis(amino acid)–Cu(II) complex. By choosing a more efficient column and the proper mobile phase, we have further improved the separation of Dns-amino acids. The present system is more efficient and faster. We achieved the baseline resolution of a group of amino acids that possess small alkyl substitutents with and without polar groups (Figs. 1 and 2), in addition to the amino acids we had previously resolved. The retention and selectivity of these smaller amino acids are very sensitive to the acetonitrile concentration, because the organic modifier influences the distribution of the metal complexes on the hydrocarbonaces stationary phase.

It is of interest that D-serine eluted before the L-isomer. This elution order represents the reverse of what was generally observed for the other amino acids. The free hydroxyl group in serine may participate in coordination and alters the stability of the Dns-serine–Cu(II)–L-proline ternary complex.

The effect of the stationary phase on the selectivity of the amino acids was examined. Table I gives the capacity ratio (k') and the selectivity factor (α) of the Dns-

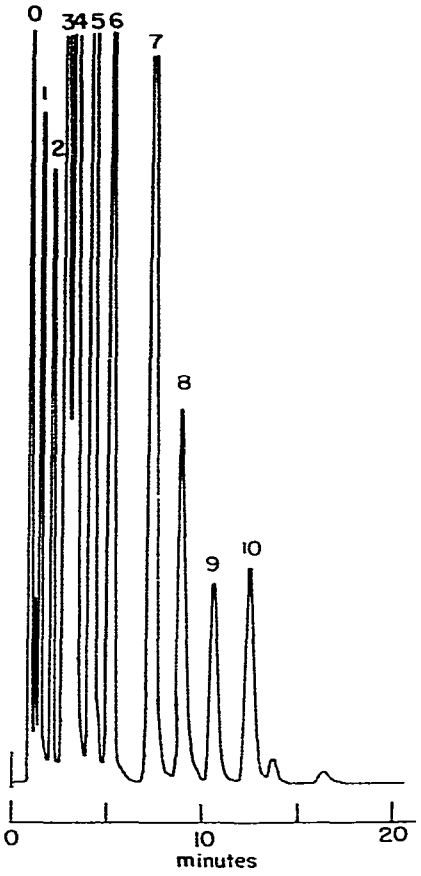

Fig. 3. Separation of L-Dns amino acids. Mobile phase: 20% acetonitrile in an aqueous solution containing 5 m*M* L-proline. 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 0.5 g ammonium acetate/l of deionized water. pH 7.0. Column: 15.0 × 0.42 cm Spherisorb C_{18}. Flow-rate: 2.0 ml/min. 0 = Reagent; 1 = hydroxyl proline; 2 = serine and threonine; 3 = alanine and α-amino butyric acid; 4 = proline; 5 = valine; 6 = norvaline and methionine; 7 = leucine; 8 = norleucine and phenylalaine (L); 9 = tryptophan; 10 = phenylalanine (D).

amino acids on two reversed-phase packings, LiChrosorb RP-8 and Spherisorb C_{18}. Because the carbon content and chain length on these materials are different, the acetonitrile concentration in the mobile phases was different. It is evident from the k' values that the selectivity between the amino acids is affected by the choice of stationary phases. However, on examination of the stereoselectivity between the D- and L-amino acids, the α values for the same amino acid are in reasonable agreement between the two systems suggesting that the stereoselectivity is independent of the packing used. Thus, the separation of D- and L-amino acids in the two systems occurs by the same mechanism; namely, the formation of Cu(II) ternary complexes with L-proline and the Dns-amino acids, with separation of the ternary complexes on the stationary phase. The selectivity factor which represents the difference in thermodynamic stability of the isomeric metal complexes is constant.

The metal complex system is highly selective. An example of the separation of

TABLE I

CAPACITY RATIO (k') AND SELECTIVITY (α) OF D- AND L-Dns-AMINO ACIDS ON TWO DIFFERENT COLUMN PACKINGS

Column: 15.0 × 0.42 cm. Flow-rate: 2.0 ml/min. LiChrosorb RP-8: Mobile phase: 20% acetonitrile (unless otherwise indicated) in an aqueous solution containing 5 m*M* L-proline, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 0.5 g ammonium acetate/l of deionized water. pH 7.0. Spherisorb C_{18}: Mobile phase: 15% acetonitrile in an aqueous solution containing 5 m*M* L-proline, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 0.5 g ammonium acetate/l of deionized water. pH 7.0.

Amino acid	*Abbreviation*	*LiChrosorb RP-8*			*Spherisorb C_{18}*		
		k'_L	k'_D	α	k'_L	k'_D	α
Serine	Ser	23.0*	20.0*	0.87	3.7	3.2	0.87
Threonine	Thr	19.0*	31.2*	1.6	3.7	4.6	1.23
Alanine	Ala	4.1	4.3	1.1	5.7	6.6	1.2
α-Aminobutyrate	α-AB	5.4	6.6	1.2	7.3	9.2	1.2
Valine	Val	7.7	10.1	1.3	11.4	15.0	1.3
Methionine	Met	9.7	12.3	1.3	11.4	14.8	1.3
Norvaline	N-Val	10.1	13.7	1.4	14.6	19.0	1.3
Isoleucine	I-Leu	14.7	19.7	1.3	—	—	—
Leucine	Leu	17.0	25.4	1.5	23.9	32.6	1.4
Norleucine	N-Leu	22.1	31.2	1.4	32.6	45.7	1.4
Phenylalanine	Phe	17.4	29.2	1.7	32.6	52.8	1.6
Tryptophan	Trp	23.0	43.4	1.9	41.2	71.2	1.7

* 10% acetonitrile.

many L-Dns-amino acids is shown in Fig. 3. Both D- and L-phenylalanine are included in this chromatogram to demonstrate the efficiency and selectivity of the present separation. The system can be optimized for the separation of natural amino acids.

REFERENCES

1 S. Lam and F. K. Chow, *J. Liquid Chromatogr.*, 3 (1980) 1579.
2 S. Lam, F. Chow and A. Karmen, *J. Chromatogr.*, 199 (1980) 295.