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Communications to the Editor

Reversed Phase Chromatographic Resolution of Amino Acid Enantiomers with Metal–Aspartame Eluants

Sir:

The resolution of amino acid enantiomers is of importance in peptide synthesis and structure determinations. While such methods as selective crystallization and enzyme degradation have been used, they are of limited use, frequently owing to their specificity. Chromatographic methods of analysis offer the possible advantage of resolving not only a pair of enantiomers but also a mixture of several amino acid enantiomers. Gas chromatographic separation of D- and L-amino acids has been described by, among others, Gil-Av,[1] Feibush,[2] and Bayer,[3] who have used chiral stationary phases. The method, however, requires the derivatization of the amino acids to more volatile compounds suitable for GC separations. For this reason, modern high performance liquid chromatography (HPLC) can be a more attractive tool for enantiomeric resolutions. Several approaches have been advanced. Davankov and his co-workers[4] and Lefebvre et al.,[5] to name a few, have used ligand exchange chromatography. There the separation of the enantiomers was achieved with an α-amino acid–copper(II) complex grafted onto a resin. Frequently the grafted amino acid of choice is proline, although, as Angelici[6] has shown, other amino acids can form stereoselective complexes. Cram and his group[7] have used chiral crown ethers bonded to chromatographic support for the separation of the optical isomers. A similar approach was reported by Blasius.[8] More recently, Hara[9] has reported the separation of the enantiomers of N-protected amino acid esters on L-valyl derivatives bonded to silica gel. Pirkle[10] has demonstrated the resolution of 3,5-dinitrobenzoyl derivatives of amino acids on chiral fluoro alcohols. Gaal and Inczedy[11] as well as Yoneda and Yoshizawa[12] have utilized optically active Co(III) compounds to achieve optical resolution. In an entirely different approach, Karger and his group[13] have used the zinc(II) complex of L-2-alkyl-4-octyldiethylenetriamine in an aqueous mobile phase to obtain the resolution of the densyl derivatives of amino acids. Hare and Gil-Av[14] have reported very recently the use of a proline–copper(II) complex in an aqueous mobile phase as the resolving reagent in the ion-exchange separation of D- and L-amino acids.

The work described above, while augmenting greatly the arsenal of the chemist, suffers from such disadvantages as (a) applicability to only one or two pairs of amino acids, (b) the need for derivatized amino acids, and (c) harsh conditions such as high temperature. We report here preliminary results of a chromatographic separation which has the potential of eliminating most of these restrictions.

To resolve optical isomers directly by chromatography, diastereomers must be formed in situ while the resolution takes place. To form the diastereomers, a chiral reagent can be introduced either to the mobile or the stationary phase. Since the mobile phase is inherently easier to manipulate, we prefer to add the chiral reagent to it, in accordance with the approach of Karger[13] and of Hare and Gil-Av.[14] Like other workers we have utilized the fact that amino acids form complexes with metal cations and that the stability of the isomers can be stereodependent (viz., ref 6 and 15 and references therein). We have chosen the metal complex of the dipeptide L-aspartyl-L-phenylalanine methyl ester as the resolving agent. The rationale for choosing this particular dipeptide is as follows. (a) This dipeptide is available commercially under the tradename

Table I. The Capacity Ratios k' and Selectivity Factors α[a] of Some Amino Acid Enantiomers as a Function of the Acetonitrile (ACN) in the Mobile Phase. The Mobile Phase Contains 10^{-3} M Copper–Aspartame Complex

solute	0% ACN		7% ACN		8% ACN		10% ACN	
	k'	α	k'	α	k'	α	k'	α
L-Dopa	3.2	1.5						
D-Dopa	4.8							
L-tyrosine	5.4	1.6	1.2	1.5	1.0	1.7		
D-tyrosine	8.9		1.8		1.7			
L-phenylalanine	*b*		4.8	1.6	4.3	1.3		
D-phenylalanine	*b*		7.8		5.4			
L-tryptophan	*b*		*b*		13.2	1.3	5.6	1.2
D-tryptophan	*b*		*b*		16.7		6.8	

[a] See note 18. [b] Retention times too long for accurate measurements.

Table II. The Capacity Ratios k' and Selectivity Factors α[a] of Some Amino Acid Enantiomers as a Function of the Concentration of the Zinc–Aspartame Complex in the Mobile Phase

solute	10^{-3} M		5×10^{-4} M		2.5×10^{-4} M	
	k'	α	k'	α	k'	α
L-tyrosine	1.9	1.3	2.9	1.4	5.3	1.5
D-tyrosine	2.5		4.2		7.9	
L-phenylalanine	5.5	1.6	9.3	1.7	16	1.9
D-phenylalanine	9.0		16.0		30	
L-tryptophan	19.4	1.2	34	1.2	*b*	
D-tryptophan	23.0		42		*b*	

[a] See note 18. [b] Retention times too long for accurate measurements.

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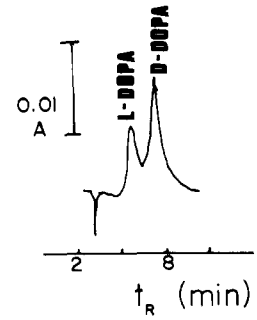

Figure 1. Separation of DL-Dopa on a reversed phase column. The mobile phase is H_2O with 10^{-3} M Aspartame and 10^{-3} M Cu(II). Chromatographic conditions are given in note 17.

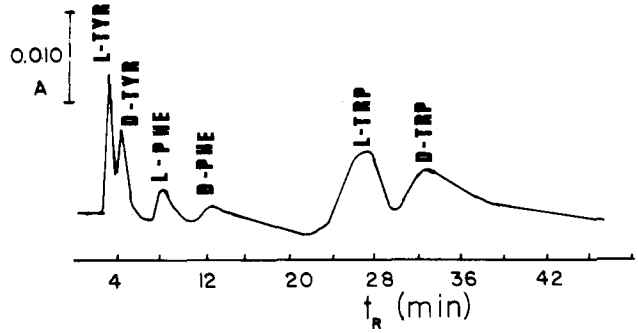

Figure 2. Separation of DL-tyrosine, DL-phenylalanine, and DL-tryptophan on a reversed phase column. The mobile phase is H_2O with 5×10^{-4} M Aspartame and 5×10^{-4} M Zn(II). Chromatographic conditions are given in note 17.

Aspartame, which is an artificial sweetener. (b) The presence of the free α-amino and β-carboxyl groups on the aspartyl residue and a blocked carboxyl group on the phenylalanine residue means that the metal ion is complexed most likely by the aspartylamine and β-carboxyl groups, resulting in a six-membered ring as suggested by Touche and Williams.[16] The ring is less stable than the five-membered ring typical of an α-amino acid-metal complex, allowing the amino acids to be separated more easily from the metal ion. (c) The phenyl group can facilitate the separation via hydrophobic interactions with one isomer of the DL pair. (d) The hydrophobic phenyl and methyl ester moieties allow separation on the usual reversed phase column.

Our intial attempt used a mobile phase consisting of 10^{-3} M Aspartame and Cu(II) in various water-acetonitrile mixtures. DL-tryptophan, DL-phenylalanine, DL-tyrosine, and DL-Dopa were injected into the chromatograph.[17] Table I shows the results of this study. The large α values,[18] which indicate the selectivity of the system, should be noted. As expected, increasing the amount of the organic modifier, acetonitrile in this case, shortens the analysis time. To elute tryptophan in a reasonable time, at least 5% acetonitrile had to be added to the mobile phase. Under all conditions, the L isomer eluted before the D.

To overcome the high detector background signal due to the complex in the mobile phase we have substituted Zn(II) for Cu(II). Table II shows that zinc-Aspartame in the mobile phase can resolve the enantiomers quite successfully. The increase in the retention times and in the selectivities as the amount of zinc(II)-Aspartame is decreased should be noted. A possible explanation of this phenomenon is as follows. If the stationary support is saturated with zinc-Aspartame complex, then the amount of that complex in the mobile phase controls the elution. When the amount of zinc(II)-Aspartame in the mobile phase is decreased, the concentration of the complex adsorbed on the reversed phase increases relative to that in the eluant and the retention time lengthens. This point should be checked further. The retention order is as above: the L isomers elutes before the D. A comparison of Tables I and II shows that, under equivalent conditions, the retention times are longer with the Cu(II) complex.

The examples of the separations are shown in Figures 1 and 2. The former shows the separation of Dopa isomers using copper-Aspartame. Figure 2 demonstrates the separation of the isomers of tyrosine, phenylalanine, and tryptophan with zinc-Aspartame. The chromatographic efficiencies, especially for the strongly retained compounds, are not very good. However, no attempts were made to optimize the system. Some very preliminary results with buffers indicate that the efficiencies can be improved greatly.

Further work in progress shows that the enantiomers of all of the hydrophobic and some polar amino acids can be separated.

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(17) The chromatographic conditions were as follows: a Spectra Physics chromatograph Model 8000 was used; the column was an ODS one, 25 cm long; the mobile phase flow rate was 2 mL/min; column temperature was 32 °C; detection was done at 275 or 254 nm.
(18) The selectivity factor α is defined as the ratio of the partition coefficients of the solutes of interest; the capacity factor k' is defined as the amount of the solute in the stationary phase proportional to that in the mobile phase. It is directly proportional to the partition coefficient. Large k' values indicate long retention times.

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Preparation and Characterization of an Oxoporphinatochromium(V) Complex

Sir:

Oxometalloporphyrin species have been implicated as intermediates in the catalytic cycles of peroxidases[1] such as horse radish peroxidase and monooxygenases such as cytochrome P-450.[2] Although simple oxometalloporphyrin complexes of vanadium(IV)[3] and molybdenum(V)[4] are known, these compounds do not undergo the oxygen-transfer reactions characteristic of these enzymes. Recently, we reported that chlorotetraphenylporphinatoiron(III) was capable of cata-

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