# Stereoselectivity in the Ternary Complexes Copper(II)–*N*-Benzyl-L-proline–D- or L-α-Amino-acids

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Chromatographic elutions of D,L-phenylalanine, D,L-proline, and D,L-histidine have been made on a copper-loaded polystyrene resin containing L-proline groups. The isomers of D,L-phenylalanine were not separated while D,L-proline and D,L-histidine could be completely resolved, the order of elution of the enantiomers being reversed. Ternary complexes, models of the associations occurring in the chromatographic process on the active sites of the resin, have been studied in aqueous solution. Formation constants of the ternary species copper(II)-*N*-benzyl-L-proline-D- or L-amino-acid have been calculated from potentiometric curves with the aid of the MINIQUAD program. The values allow us to explain the chromatographic results since stereoselectivity is significant only in the mixed systems containing proline and histidine. Suggestions are given for the structures of all the ternary complexes.

MUCH work has been devoted, over the past few years, to the direct resolution of racemic compounds by liquid chromatography. The separation of optical isomers of α-amino-acids has been of particular interest to many workers. In this field, ligand-exchange chromatography using optically active phases has been frequently mentioned as a method giving very efficacious separations.[1-6] The results and in particular the elution orders of the enantiomers could occasionally be explained by stereoselective interactions in solution on the formation of ternary complexes involving the cation of a transition metal, an L- or D-α-amino-acid, and an optically active molecular model of the active site of the chromatographic support. That was how Snyder and Angelici [7] pointed out that some *N*-methoxycarbonyl-L-α-amino-acids are more associated to α-amino-acids of L configuration rather than D configuration in the presence of copper(II) cations. Davankov and Mitchell [8] showed that *N*-benzyl-L-proline, a model molecule of their polystyrene resins, has more affinity for D-proline and D-valine than for their corresponding L isomers.

The present report deals with potentiometric studies in aqueous solution with the intention of determining the stability constants of the ternary complexes formed between the copper(II) cation, *N*-benzyl-L-proline, and an L- or D-α-amino-acid. The work was limited to the case of three α-amino-acids: phenylalanine (phe), proline (pro), and histidine (his). They were chosen on account of the different results obtained when direct resolutions of the racemic forms were attempted by ligand-exchange chromatography on a polystyrene resin loaded with copper(II) ions and containing L-proline as the fixed ligand. The preparation [9] and properties [6] of the stationary phase have been described. It was shown that D-proline has a considerably higher retention time than L-proline. Recently, an inverse elution order was found for the isomers of D,L-histidine.[10,11] Under the same chromatographic conditions, no significant resolution of racemic phenylalanine was observed.

## EXPERIMENTAL

*Reagents.*—The α-amino-acids were obtained from Fluka (puriss grade). The styrene–divinylbenzene copolymer with 1% crosslinkage (Biobeads S-Xl, 200—400 mesh) was from Bio.rad Labs. The resin was treated as previously described [9] so that it contained fixed L-proline ligands complexed by copper(II).

The amount of fixed L-proline ligands in the asymmetric resin was determined by potentiometric titration. A limited capacity value of 1.53 milliequivalents of L-proline per gram of dry resin was chosen so that very few broken beads were produced. Copper(II) ions were chelated to the resin to an extent of 90% of the theoretical capacity calculated on the basis of one copper(II) ion bound to two fixed L-proline ligands.

*N*-Benzyl-L-proline was prepared according to a method similar to that previously described.[12] To ethanol (60 $cm^3$) containing benzyl chloride (Fluka, puriss grade) (6.60 $cm^3$, 0.057 mol) were added L-proline (6 g, 0.052 mol) and sodium hydroxide (6.5 g, 0.162 mol) dissolved in water (40 $cm^3$). The boiling mixture was stirred for 2 h and then extracted by steam for 6 h in order to remove benzyl alcohol. The solution was acidified to pH 5.9 by adding acetic acid (8 $cm^3$) and evaporated *in vacuo*. The residue was dissolved in chloroform (150 $cm^3$) which was evaporated after filtration. The excess of acetic acid was eliminated by treating the oily material several times with portions (100 $cm^3$) of dry toluene which were then evaporated. The residue was treated with ethyl acetate and dissolved in the minimum volume of absolute alcohol. Crystallization, initiated by adding a small amount of dry diethyl ether, occurred in the cold mixture. Yield: 5.4 g (51%) of pure neutral *N*-benzyl-L-proline, m.p. 164 °C, α (293 K, 589 nm) = $-28.4°$ $dm^{-1}$ $(g\ cm^{-3})^{-1}$ ($c$ = 0.01 g $cm^{-3}$) in absolute ethanol.

*Chromatographic Experiments.*—The liquid chromatography system consisted of a Waters Assoc. 6 000 A pump and U6K injector. Two detection cells were kept at 25 °C and used in series so that both the differential refractive index and the optical rotation of the eluate could be recorded. The resin was packed in a column of length 220 mm and internal diameter 7.4 mm.

*Potentiometric Measurements.*—The potentiometric titrations were performed by a Tacussel automatic titrator involving the Titrimax equipment TT100 and TT300. An electronic burette monitored by the Titrimax allowed the addition, at equilibrium, of incremental volumes of a carbonate-free sodium hydroxide solution (0.1 mol $dm^{-3}$) to an aqueous solution (50 $cm^3$) containing sodium nitrate (5.0 mmol) as an ionic background, an α-amino-acid (0.1 mmol) or an equimolar mixture of an α-amino-acid (0.05

mmol) and *N*-benzyl-L-proline (0.05 mmol), nitric acid (0.1 mmol), and a variable volume of copper(II) nitrate. A standardized glass electrode was carefully calibrated in terms of hydrogen-ion concentrations. The titration curves obtained at 25 °C under nitrogen gave the data which were used for the determination of the stability constants of the various complexes. Calculations were made with the aid of the MINIQUAD program [13] which was run on an IBM 168 computer.

## RESULTS AND DISCUSSION

*Chromatographic Elutions.*—In order to reduce the retention times of the three α-amino-acids an eluant with a displacing ligand was used. D,L-Proline was eluted using a 1 N aqueous solution of ammonia (1 mol $dm^{-3}$) at a rate of 12 $cm^3$ $h^{-1}$ (Figure 1). The same eluant was used for D,L-histidine elutions at a rate of 30 $cm^3$ $h^{-1}$ (Figure 2). The ability of a stationary phase to resolve racemic compounds is conveniently expressed by separation factors (α) given by equation (1) where $V_M$ is the

$$\alpha = \frac{V_{RB} - V_M}{V_{RA} - V_M} \qquad (1)$$

free volume and $V_{RB}$ and $V_{RA}$ are the retention volumes of the two isomers A and B, B being the last eluted component. It was found that in spite of the large width of the peaks, D,L-proline and D,L-histidine could be completely resolved into their enantiomers because of the high values of their separation factors. D-Proline is more retained than L-proline with an α value of 2.3; a reverse elution order is observed for histidine with an α value of 2.9. Under the same chromatographic conditions, D- and L-phenylalanine were separately introduced into the column since two peaks could not be defined at the elution of the racemic form. For the two isomers, the peaks are very broad having approximate maxima at an elution volume of 50 $cm^3$, and it was concluded, therefore, that no significant resolution occurred.

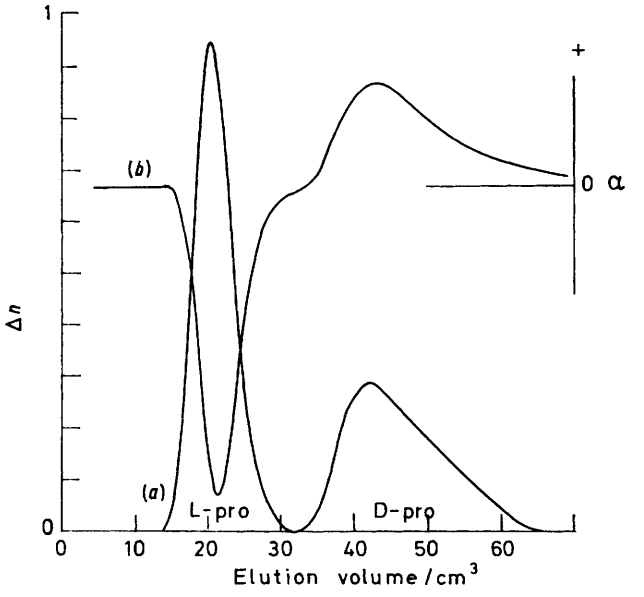


FIGURE 1 Elution of 3.5 mg of D,L-proline by 0.94 mol $dm^{-3}$ $NH_3$ at a flow rate of 12 $cm^3$ $h^{-1}$. (*a*) Differential refractive index, Δ*n*; (*b*) rotation power α (both in arbitrary units)

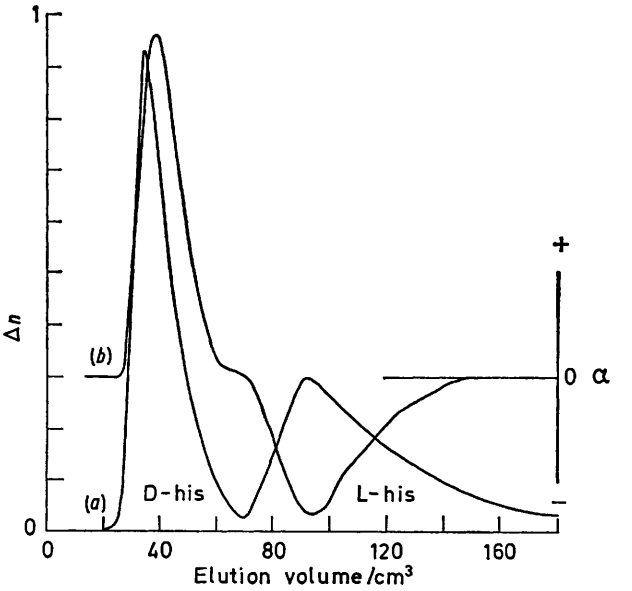


FIGURE 2 Elution of 20 mg of D,L-histidine by 0.98 mol $dm^{-3}$ $NH_3$ at a flow rate of 30 $cm^3$ $h^{-1}$. Details as in Figure 1

*Potentiometric Determinations.*—Four variables *w*, *x*, *y*, and *z* were used for convenience as subscripts for the formation constants $\beta_{wxyz}$ of the complexes with general formula $Cu_wL_x(bpro)_yH_z$ where Cu represents the copper(II) ion, H the proton, L an α-amino-acid, and bpro the *N*-benzyl-L-proline molecule. The formation constants $\beta_{wxyz}$ were calculated according to relation (2) where [Cu], [L], [bpro], and [H] are the concentrations

$$\beta_{wxyz} = \frac{[Cu_wL_x(bpro)_yH_z]}{[Cu]^w[L]^x[bpro]^y[H]^z} \qquad (2)$$

of the free species in solution; $[Cu_wL_x(bpro)_yH_z]$ represents the concentration of the complex. The acidity constants of *N*-benzyl-L-proline, phenylalanine, proline, and histidine are reported in Table 1.

For each α-amino-acid, five titration curves, were obtained at five different ratios (0.25, 0.35, 0.50, 0.70, and 0.95 : 1) of copper (II) ions to α-amino-acid molecules. The five curves were limited to pH values <7.5 and occasionally <6.7 when precipitation occurred. A set of about 150 experimental points taken from the five

TABLE 1

Formation constants (log $\beta_{wxyz}$) for the binary complexes of α-amino-acids with copper(II). Standard deviations are given in parentheses

| *w* | *x* | *y* | *z* | bpro | *w* | *x* | *y* | *z* | phe (L or D) | pro (L or D) | his (L or D) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 1 | 1 | 9.95(1) | 0 | 1 | 0 | 1 | 9.15(1) | 10.69(1) | 9.18(1) |
| 0 | 0 | 1 | 2 | 11.89(3) | 0 | 1 | 0 | 2 | 10.96(3) | 12.59(2) | 15.38(2) |
| | | | | | 0 | 1 | 0 | 3 | | | 16.76(3) |
| 1 | 0 | 1 | 0 | 6.97(1) | 1 | 1 | 0 | 0 | 7.62(1) | 8.99(1) | 10.37(1) |
| | | | | | 1 | 1 | 0 | 1 | | | 14.42(1) |
| 1 | 0 | 2 | 0 | 12.84(2) | 1 | 2 | 0 | 0 | 14.22(2) | 16.29(2) | 18.07(2) |
| | | | | | 1 | 2 | 0 | 1 | | | 24.17(2) |
| 1 | 0 | 1 | −1 | −0.26(4) | 1 | 1 | 0 | −1 | 0.51(4) | 1.60(3) | 2.80(2) |

curves was used for the calculation of the formation constants of the binary complexes (Table 1). The calculated values are generally close to results reported in the literature; as stated previously, no stereoselectivity was detected. The (1, 0, 1, −1) and (1, 1, 0, −1) hydroxy-species were considered in order to account for the shape of the titration curves of copper–proline and copper–histidine mixtures for copper : amino-acid ratios >0.5 : 1. Under these conditions, appreciable concentrations of hydroxo-species were calculated above pH 6.5. The inclusion of similar species in binary mixtures involving *N*-benzyl-L-proline or L-phenylalanine was made by analogy with the other two preceding binary mixtures, although there is no direct evidence that these species exist, since precipitation occurred in the pH region where their concentrations are expected to be appreciable. In general, hydroxo-species were of minor importance and did not have a large influence on the formation constants of the major species or on the statistics of the computed solution.

Subsequent calculations were made to determine the formation constants of the ternary species (Table 2) formed with copper(II) ions, *N*-benzyl-L-proline, and the L or D isomer of any of the three α-amino-acids proline, histidine, and phenylalanine. For each isomer, four titration curves were run for four different values (0.35, 0.40, 0.60, and 0.70 : 1) of the ratio of copper(II) ions to the total number of moles of *N*-benzyl-L-proline and α-amino-acid. About 120 experimental points were taken from the four curves and subsequently used for the refinement process. The pH values were limited to 7.5 unless a lower limitation was made necessary on account of precipitation. Two ternary species were formed, the (1, 1, 1, 0) species, in all cases, and the protonated complexes (1, 1, 1, 1) when histidine was involved in a ternary mixture.

From the values of the constants reported in Table 2, large stereoselective effects are evident in the formation of ternary complexes involving L- or D-histidine and L- or D-proline. On the other hand, no stereoselectivity is observed in the case of L- or D-phenylalanine. The calculated values agree well with the chromatographic results. The complete resolution of racemic proline and histidine is thus due to stereoselective effects as observed in the soluble ternary species, which are models, of the associations occurring in the ligand-exchange chromatography. For these two α-amino-acids the chemical nature of the chromatographic support has probably no determining influence on the separation process, provided the access of solutes to the active sites has been made possible.

TABLE 2

Formation constants (log $\beta_{wxyz}$) for the ternary complexes formed between copper(II), *N*-benzyl-L-proline and an enantiomer of proline, histidine, or phenylalanine. Standard deviations are given in parentheses

| | L-pro | D-pro | L-his | D-his | L-phe | D-phe |
|---|---|---|---|---|---|---|
| log $\beta_{1110}$ | 15.18(5) | 15.57(3) | 16.91(2) | 16.17(4) | 14.17(3) | 14.14(2) |
| log $\beta_{1111}$ | | | 22.60(6) | 22.51(8) | | |

Suggestions for the structures of the ternary complexes can be made from the constants given in Table 2 and the use of Dreiding molecular models. In the case of complexes (Cu, L- or D-pro, bpro), a *trans* conformation around the copper(II) ion is more likely than a *cis* conformation which would be sterically hindered because of substitution on the two nitrogens. Consequently, the mixed complex containing L-proline is less stable than its diastereoisomer involving D-proline in which the two proline rings are placed on both sides of the copper co-ordination plane.

It is more difficult to suggest the structures of the ternary species containing an L- or D-histidine ligand since histidine may co-ordinate in various geometric arrangements. For the monoprotonated complexes (1, 1, 1, 1), it was previously [14] suggested that the mode of bonding of bidentate histidine is glycine-like with a protonated imidazolium group. In the copper co-ordination plane, the conformation will be *trans* in such a system, since *N*-benzyl-L-proline has a tertiary nitrogen. From molecular models made accordingly, it is apparent that stereoselectivity is probably absent in these complexes. This may explain the very close results (22.60 and 22.51) (Table 2) for the two log $\beta_{1111}$ values. With neutral ternary species (1, 1, 1, 0), one possible structure gives a larger stability to the complex containing L- rather than D-histidine. In this structure, tridentate L-histidine is bonded glycine-like to copper(II) in the co-ordination plane, the imidazole nitrogen interacting weakly in an axial position. Two reasons can be put forward to support the assumption of such a geometry. First, a histamine-like bonding of histidine to copper(II) as previously stated for binary [14-16] and ternary [17,18] complexes is unlikely owing to the bulky tertiary nitrogen of *N*-benzyl-L-proline. Secondly, the two acidity constants of the ternary complexes with L- or D-histidine, which can be calculated from the constants in Table 2, are close to the acidity constant of the imidazolium group of histidine whether bound or not to copper(II).

For the last type of ternary complexes containing D- or L-phenylalanine, the absence of a significant difference in the formation constants (log $\beta_{1110}$) shown in Table 2 can be explained in terms of the structure of the complex: the conformation around the copper(II) ion is assumed to be *trans* similarly to the other complexes. Molecular models indicate that stereoselectivity is unlikely to be expected since the two ligands *N*-benzyl-L-proline and phenylalanine, either L or D, do not interact.

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