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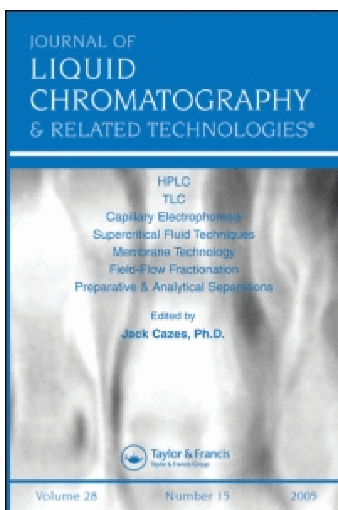

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Ligand Exchange Chromatography of Cephalosporin C on Polystyrene Resins Containing Copper Complex of Lysine Derivatives

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LIGAND EXCHANGE CHROMATOGRAPHY OF CEPHALOSPORIN C ON POLYSTYRENE RESINS CONTAINING COPPER COMPLEX OF LYSINE DERIVATIVES

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ABSTRACT

A ligand-exchange chromatography procedure for the separation of cephalosporin C from an artificial mixture is described. Three new sorbents were synthesized by immobilizing the ligands ε-L-lysine,glycyl-ε-L-lysine and diglycyl-ε-L-lysine on a polystyrene matrix(SX 1 Bio-Rad).These resins were loaded with Cu(II)as a complexing agent.A good resolution of cephalosporin C was achieved only using the ε-L-lysine resin complexed with copper. Models of copper complexes involved are proposed, for the chromatographic separation of cephalosporin C.

INTRODUCTION

Cephalosporin C,the starting material for the synthesis of various antibiotics,is usually extracted from its biosynthesis medium by means of adsorption(1,2,3) or ion exchange(4) chromatography and this extraction is not always efficient because of the low selectivity of the sorbents usually employed.

In a previous study(5),we successfully purified cephalosporin C by using new selective sorbents.These sorbents were obtained by immobilizing three ligands(ε-L-lysine,glycyl-ε-L-lysine,and diglycyl-ε-L-lysine) on a polystyrene matrix(Bio-Beads S X_1).The best

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results were achieved with the ε-L-lysine resin.However the selectivity of these sorbents depends greatly on the concentration of salt(NaCl)in the eluent solution. To overcome this drawback,the three resins were complexed with copper ions,when the binding of solutes to the copper-complexed ligands was not influenced by the presence of inorganic salts.

This paper describes a ligand-exchange chromatography procedure for the separation of cephalosporin C from by-products,which are usually found in its biosynthesis medium. The composition and the features of the eluent were varied in order to optimize the procedure.

EXPERIMENTAL

Reagents

The aminoacids,organic acids and sodium acetate were obtained from Fluka(Switzerland).The dipeptide glycylglycine was purchased from Bachem(Switzerland).The divinylbenzene-crosslinked polystyrene(Bio-Beads SX1,2% divinylbenzene,particle size 200-400 mesh) was obtained from Bio-Rad Labs(Richmond,California,USA).. Cephalosporin C and deacetylcephalosporin C were a gift from Roussel-Uclaf(Paris,France).The acetic acid was of reagent grade.

Synthesis

The sorbents were synthesized by a procedure described elsewhere(6).According to elemental analysis and potentiometric titration,the chemical capacities were 0.6-0.8 mmol(ε-L-lysine resin) and 0.4-0.6 mmol (glycyl-ε-L-lysine resin and diglycyl-ε-L-lysine resin)of fixed lysine per gram of dry resin. The copper complex was formed by treating the resin with aqueous acetate buffer solutions containing copper sulfate. Columns were packed by a conventional slurry method using a precolumn(7).

Instrumentation

The liquid chromatograph consisted of a Waters Model A.L.C.200 liquid chromatograph,equipped with a M 600 A pump,a M 440 UV detector with a 12.5 μl flow cell,a R 401 differential refractometer, and a U6K sample injector fitted with a 2 ml sample loop.

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Stainless steel tubing (0.95 cm ID) 30 cm long was purchased from Waters Associates. Volumes of 20 to 150 µl containing 6 mM solute were injected with a Hamilton syringe into the chromatographic system.

RESULTS AND DISCUSSION

Several buffers eluents, among those usually employed in ligand exchange chromatography(8,9,10) were tested, within the stability range of cephalosporin C towards pH(1 to 10). At pH 10, this compound undergoes a rapide inactivation, which leads to the loss of U.V. chromophore and the formation of two new titratable acid groups (11). On the other hand, at pH 1, cephalosporin C is transformed into a new substance, having antibacterial properties, namely deacetylcephalosporin C lactone. The antibiotic was irreversibly adsorbed on the three stationary phases, when it was injected in an isocratic flow with ammonium hydroxide solutions, or with ammonium phosphate buffers. On the other hand cephalosporin C was only retarded on the supports with acetate buffers as eluents. Therefore all the following experiments were performed in acetate buffers.

Isotherms of Cu(II) sorption on the three sorbents were determined by a batch process (Fig. 1-3) (12,13). It should be noted that saturation with Cu(II) corresponded to 1 mole of Cu(II) per 2 moles of ligands, suggesting the formation of Resin-Cu-Resin bidentate complex. The features of resins complexed with copper are shown in Table 1. The swelling capacity of the sorbents fits the results obtained by Davankov and coworkers (12). On the other hand it appears that the sorption capacity was not greatly influenced by the pH of the acetate buffer in the range 4 to 6.

For the optimization of the conditions of separations, the effects of the pH and ionic strength of the eluent were thoroughly investigated. The various solutes were injected separately onto the three stationary phases. Capacity factors k' were calculated using the equation $k' = \frac{Ve - Vo}{Vo}$, where Ve is the elution volume for a chromatographic peak and Vo is the column void volume.

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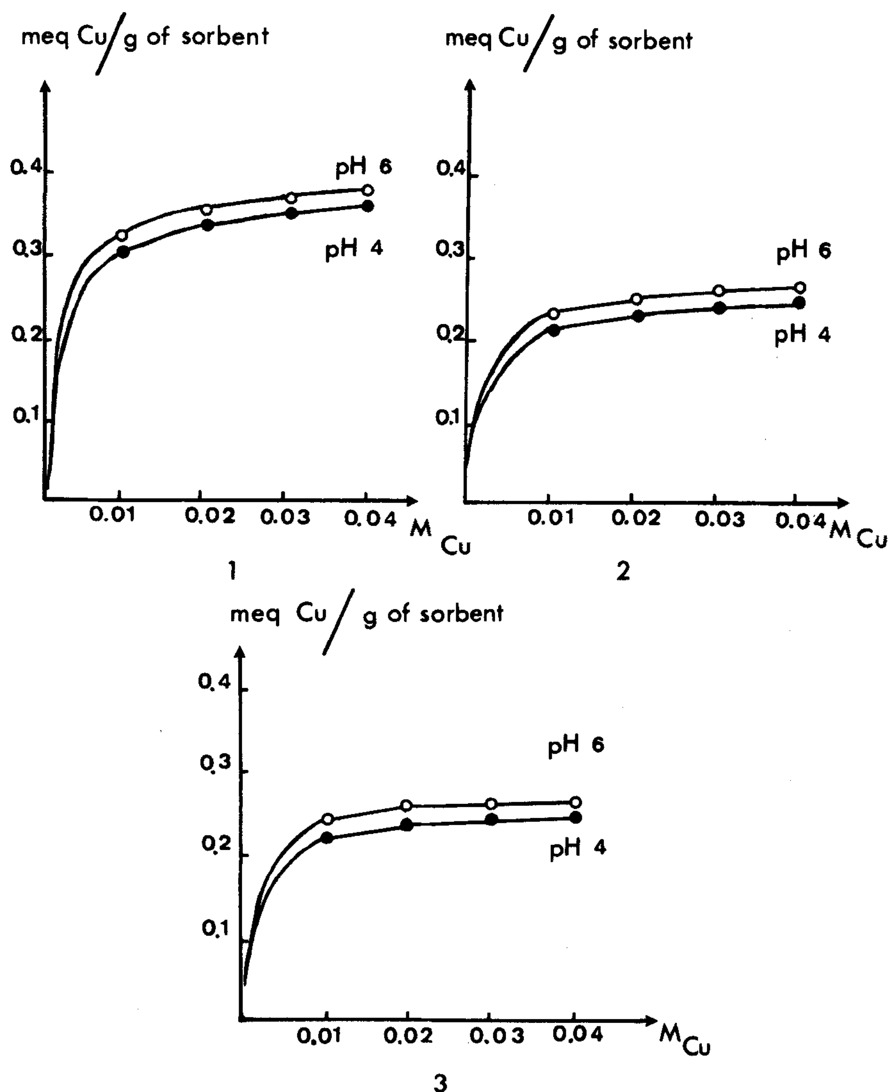

FIGURES 1-3: Adsorption isotherms of copper on the ε-L-lysine resin(1), glycyl-ε-L-lysine resin(2) and diglycyl-ε-L-lysine resin(3).
Copper adsorbed on the resin versus concentration of copper in the solution.

TABLE 1

Features of the resins in acetate buffers

Q_1 : quantity of sites per g of sorbent

Q_2 : quantity of copper per g of sorbent

S : swelling capacity is defined as the weight of water absorbed by unit weight of dry resin in 24 h.

Sorbents	Q_1	acetate concentration = 1 M			
		pH = 4		pH = 6	
	meq/g	S	Q_2 meq/g	S	Q_2 meq/g
ε-L-lysine resin	0.8	0.30	0.35	0.31	0.36
glycyl ε-L-lysine resin	0.6	0.29	0.25	0.29	0.25
diglycyl -ε-L-lysine resin	0.6	0.26	0.24	0.26	0.25

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Diglycyl-ε-L-lysine resin complexed with copper

Figure 4 showsthe chromatographic peak corresponding to the elution of cephalosporin C. At all pH values and ionic strengths of the acetate buffer the antibiotic was eluted partly in the void volume,and in all cases the chromatographic peak obtained was very broad.This proves that copper-complexed diglycyl-ε-L-lysine resin is not very selective for cephalosporin C.

On the other hand, a comparison of selectivity factors of amino acids(Table 2)shows that their retention is due only to the participation of a carboxyl function in the side chain(Asp,Glu) in the copper complex or to weak hydrophobic interactions(Met,Phe). The structure of the copper complex on the stationary phase could explain these results.The diglycyl-ε-L-lysine ligand probably in-

TABLE 2

Capacity factors obtained on the diglycyl-ε-L-lysine-copper resin in acetate buffers of various concentration at pH 4.

* Solutes of both L and D configurations had identical k' values

** C=acetate concentrations.

*** Peaks too wide for the elution volume to be determined

Solutes*	k'		
	C = 0.25 M	C = 0.5 M	C = 1 M
Lysine	0	0	0
Glycine	0	0	0
Alanine	0	0	0
Methionine	0.09	0.09	0
Glutamic acid	0.95	0.45	0
γ-methyl glutamate	0	0	0
Aspartic acid	1.70	0.90	0.45
Phenylalanine	0.20	0.20	0.20
Cephalosporin C	***	***	***
Deacetylcephalosporin C	***	***	***

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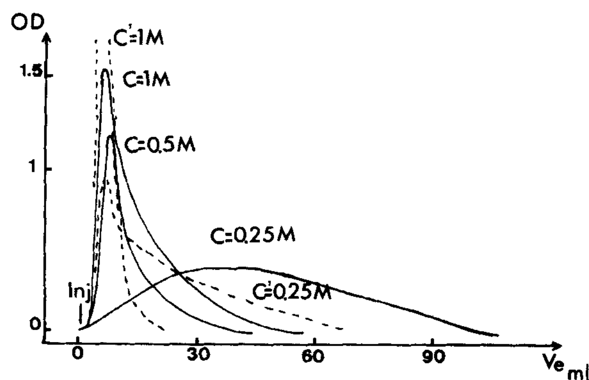

FIGURE 4 : Retardation of cephalosporin C on the diglycyl-ε-L-lysine-copper resin in acetate buffers of various concentrations at pH6 (C',---) and at pH 4 (C,——).

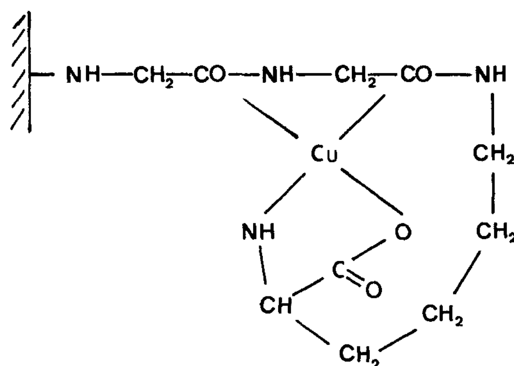

FIGURE 5 : Theoretical structure of the diglycyl-ε-L-lysine copper complex.

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teracts in the copper coordination plane with the cation as shown in Figure 5.In such a case,solutes are only able to interact with the cation in an axial position. Thus,this structure does not favour the participation of the two functions of the solutes α-amino acid moiety in the mixed complex.As a matter of fact,the proximity of the carboxyl and amine functions does not allow the coordination of one of these,with the copper complex.In contrast,the carboxylic function of aspartic and glutamic acids,located on the lateral chain,could lie on the axis of the copper complex.Such a structure,which is in good agreement with molecular models,is consequently consistent with our results.

Glycyl-ε-L-lysine resin complexed with copper

As illustrated in Table 3,cephalosporin C was retarded more strongly than α-aminoacids on this stationary phase.The highest capacity factor for cephalosporin C was obtained at pH 4,and a decreased acetate concentration resulted in an increased retention. However the capacity factor of deacetylcephalosporin C varied similarly,and the resolution factor calculated between this compound and cephalosporin C was always less than 0.5 .Therefore the use of

TABLE 3

Capacity factors obtained on the glycyl-ε-L-lysine resin complexed with copper in sodium acetate buffers.

*C = acetate concentrations, ** Solutes of both L and D configurations had identical k' values.

Solutes**	k'				
	pH = 4			pH = 6	pH = 7
	C=0.25M	C=0.5M	C = 1M	C=0.5M	C=0.5M
Lysine	0	0	0	0	0.10
Glycine	0	0	0	0.10	0.10
Alanine	0	0	0	0.10	0.10
Methionine	0.20	0.20	0.20	0.25	0.50
Glutamic acid	0.70	0.60	0.30	0.30	0.30
γ-methyl glutamate	0	0	0	0.10	0.10
Aspartic acid	1.20	0.90	0.40	0.45	0.45
Phenylalanine	0.30	0.20	0.20	0.60	0.80
Cephalosporin C	3.90	2.65	1.20	1.65	1.90
Deacetyl-cephalosporin C	2.90	1.80	0.80	0.90	1.10

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glycyl-ε-L-lysine resin does not make it possible to separate the antibiotic satisfactorily from its related contaminant.

On the other hand, neutral α-amino acids with hydrophobic features (Phe,Met) were the only ones to be retained on this support at pH 4. Furthermore, varying the concentration of the acetate buffer did not alter the capacity factors of these hydrophobic solutes. These facts indicate that the α-amino acid extremities of solutes do not participate in the copper complex and also that the retention of phenylalanine and methionine is due only to hydrophobic interactions. Moreover, the retention of dicarboxylic aminoacids can be attributed to an interaction between their β or γ

carboxyl function and the ligand complex.(See the retention of Asp,Glu and γ-methyl glutamate).

These results suggest that cephalosporin C interacts with the copper complex only by means of the carboxylfunction of its dihydrothiazine ring and that some additional hydrophobic interactions with the polymer matrix might increase the stability of this complex.

This fact could be explained by the structure of the copper ligand complex,if the amide function of the peptide bond can participate in the copper complex on the stationary phase.Thus the copper ion presumably coordinates both with the amide function and with the α-amino groups of lysine.In this case,one coordinate bond in the coordination plane and two in the axial position remain available. Obviously,the spatial disposition of these bonds prohibits the simultaneous interaction with copper,of the two functions of the solutes α-amino-acid moiety.As for the diglycyl-lysine resin the two functions of α-amino-acid ends are not able to interact in an axial position.Moreover the steric hindrance of the resin copper complex does not allow the coordination of one of these functions in the coordination plane.Therefore,this structure,which prohibits the participation of the solutes α-amino-acids ends in the mixed copper complex is in good agreement with our results.

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ε-L-lysine resin complexed with copper

As shown in Table 4,above pH 4 cephalosporin C was irreversibly bound to the support,indicating that the ternary complex involving ε-L-lysine,a copper ion,and cephalosporin C is very stable.
At pH 4,the difference between the capacity factor of cephalosporin C and those of the other solutes illustrates the great affinity and the good specificity which this support has for the antibiotic.

It also appears that this compound is more strongly retained than deacetylcephalosporin C and consequently that this support may be used for a good resolution of cephalosporin C from its contaminants.

TABLE 4

Capacity factors obtained on the glycyl-ε-L-lysine resin in acetate buffers.

* C = acetate concentration

**Solutes of both L and D configurations had identical k'values

***Peaks too wide for the elution volume to be determined.

Solutes**	k'				
	pH = 4			pH=6	pH=7
	C=0.25M	C=0.5M	C = 1M	C=0.5M	C=0.5M
Lysine	0	0	0	0.55	1
Glycine	0.75	0.65	0.65	0.75	1.65
Alanine	0.75	0.65	0.65	0.75	1.65
Methionine	2.15	1.85	1.40	2.10	2.60
Glutamic acid	***	2.25	1.40	3	3.85
γ-methyl glutamate	0.75	0.65	0.65	0.75	1.65
Aspartic acid	***	5.25	2	6.25	7.30
Phenylalanine	6.25	3.55	2.75	4.30	9
Cephalosporin C	31	16.50	5.70	∞	∞
Deacetyl-cephalosporin C	13	8.20	2.20	***	∞

On the other hand,for the first time neutral α-amino-acids(Ala,Gly) show an affinity towards the sorbent which is greater,if the solutes are hydrophobic(Phe,Met).The retention of such compounds is due to the participation of the α-amino acid extremities in the mixed copper complex as well as to hydrophobic interactions with the polymer matrix.

By comparing the capacity factor of phenylalanine obtained using acetate buffers at pH 4 in the 0.5-0.2 M concentration range one can determine the importance of hydrophobic interactions.Moreover, the difference between the affinity of glutamic acid and that of

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γ-methyl glutamate shows that the presence of another carboxyl function in the structure of solutes increases the stability of the ternary complex.May be such a function could lie on the axis position of the bound copper complex.

In the case of cephalosporin C and according to these results we can assume that the antibiotic coordinates the copper ion as a tridentate ligand.Its α-amino-acid extremity may interact in the copper coordination plane and the amide function in an axial position,with an additional ring copper interaction increasing the stability of the complex(14,15).For structural reasons it seems that the carboxyl function of the dihydrothiazine ring is not able to participate in the mixed copper complex.

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On the other hand,it is well known that ε-L-lysine is able to interact with a copper cation in its coordination plane only by means of its α-amino-acid extremity(16).Because of structural reasons,the interaction of two bound lysines to form a bis-copper complex is forbidden(17).Thus,the available coordinate bonds located in the coordination plane and the two axial ones may be occupied by solutes functions.Such a resin complex structure is quite consistent with our above assumptions concerning the coordination bonds of cephalosporin C with the bound lysine complex.

Separations

On the basis of difference in k' values,separations were carried out on a chromatographic system containing ε-L-lysine resin.The best results were obtained using an isocratic flow of 1 M acetate buffer at pH 4.Figure 6 shows a typical separation of cephalosporin C from a model mixture containing several α-amino acids and deacetylcephalosporin C. In this case,the antibiotic was obtained 99% pure,but it was collected in a large volume (40 ml),and was therefore very diluted. To cope with this difficulty,another technique was developed in which the acetate concentration of the buffer increased during the elution process;cephalosporin C was then collected in half the previous volume.
An example of such a separation is shown in Figure 7.

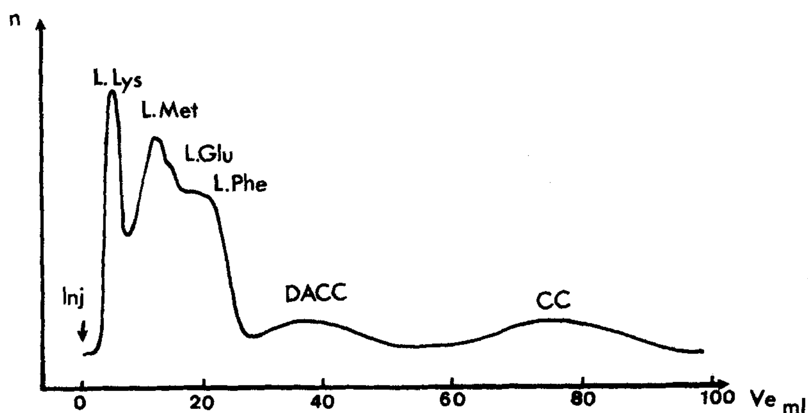

FIGURE 6 : Chromatogram of a mixture of cephalosporin C and various contaminants on the ε-L-lysine copper resin. Refractometric detection(n)
Isocratic elution with a 1M acetate buffer ,pH 4.
Flow rate 1 ml per minute . Room temperature .
Cephalosporin C = CC, Deacetylcephalosporin C = DACC.

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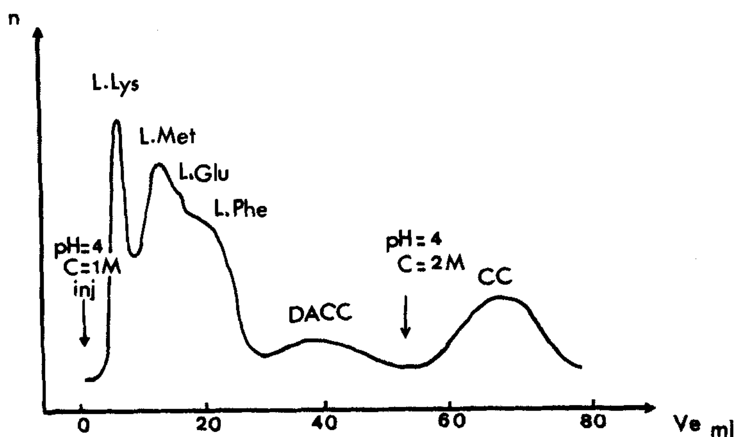

FIGURE 7 : Chromatogram of a mixture of cephalosporin C and various contaminants on the ε-L-lysine copper resin.
Elution with acetate buffers pH 4, 1M then 2M.
Refractometric detection(n) . Room temperature.
Flow rate 1 ml per minute.
CC = Cephalosporin C
DACC = Deacetylcephalosporin C.

CONCLUSION

Ligand exchange chromatography on a ε-L-lysine resin complexed with copper offers a new possibility to separate cephalosporin C from an artificial mixture of contaminants. This new sorbent has an affinity for the antibiotic which is not altered by the presence of inorganic salts(NaCl),in contrast to the results obtained using the uncomplexed ε-L-lysine resin. On the other hand,the copper glycyl-ε-lysine and copper diglycyl-ε-L-lysine ligands do not interact selectively with the antibiotic. These results can be explained by the structure of the copper complex on the stationary phases: the peptide bond present in the spacer arm can occupy some coordination bonds of the copper ion and prevent the solutes from interacting with the fixed complex. Application of the copper ε-L-lysine to a large scale separation of cephalosporin C is at present under investigation.

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REFERENCES

1) Ciba S.A.,Brevet Fr.750,292,1970.
2) Voser W.,U.S.Pat.3,725,400,1973.
3) O'Connor S.C.,Methods Enzymol.,43,296,1975.
4) Mc.Cornick S.L.,Mack.H.,U.S.Pat.3,467,654,1969.
5) Sacco D.,Dellacherie E.,J.Chromatogr.,211,79,1981.
6) Sacco D.,Dellacherie E.,Makromol.Chem.,182,763,1981.
7) Kirkland J.J.,"Chromatographie en phase liquide" Ed.Gauthier-Villars,PARIS,1972.
8) Davankov V.A.,Semechkin A.V.,J.Chromatogr.,141,313,1977.
9) Gübitz G.,Jellenz W.,Santi V.,J.Liq.Chromatogr.,4,701,1981.
10) Kurganov A.A.,Zhuchkova L.Y.,Davankov V.A.,Makromol.Chem. 180,2101,1979.

11) Morin R.B.,Jackson B.G.,Forschritte Chem.Org. Naturst. 28,343,1970.

12) Boué J.,Audebert R.,Quivoron C.,J.Chromatogr.,204 ,185,1981.

13) Foucault A.,Caude M.,Oliveros L.,J.Chromatogr.,185,345,1979.

14) Muller D.,Jozefonvicz J.,Petit M.A.,J.Inorg.Nucl.Chem.,42, 1083,1980.

15) Brookes G.,Pettit L.D.,J.Chem.Soc.Dalton,1918,1977.

16) Greenstein J.P.,Winitz M.,"Chemistry of the amino acids"Vol.1 Ed.John Wiley and Sons,New York,1961.

17) Herring R.,Trenne K.,Neske P.,J.Prakt.Chem.32,291,1966.

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