CHROM. 10,132

Note

Separation of ninhydrin-positive compounds on a single-column amino acid analyzer using lithium buffers

P. ADRIAENS, B. MEESSCHAERT, W. WUYTS, H. VANDERHAEGHE and H. EYSSEN Rega Institute, University of Leuven, Leuven (Belgium) (First received February 2nd, 1977; revised manuscript received April 7th, 1977)

During a study on the biosynthesis of penicillin¹, culture fluids and mycelial extracts of the mould *Penicillium* were examined with use of a single-column amino acid analyzer equipped with a cation-exchange column and with a lithium buffer gradient system as eluent. Since these analyses revealed the presence of many unidentified compounds, it was necessary to investigate the behaviour of a wide range of known materials. Although several papers²⁻⁹ describe the separation of some amino acids using lithium buffers, extensive standardizations such as those reported for sodium buffers^{10–12} are not available. Therefore, the elution behaviour of 145 nin-hydrin-positive substances has been compared for two Chromobeads type-B resins of different lot numbers. Since most of these compounds are naturally occurring amino acids, the results should be of interest in the analysis (with lithium buffers) of other complex biological fluids, *e.g.*, human urine or plant extracts.

MATERIALS AND METHODS

Amino acids

Standard solutions of 18 and 38 amino acids (each 2.5 μ mole/ml; Technicon, Brussels, Belgium) were used. S-Carbamylcysteine¹³, α -aminoadipic acid¹⁴, β -methoxyvaline and allo-O-methylthreonine¹⁵, β -hydroxyvaline¹⁶, hydroxypipecolic acid¹⁷, *threo-* and *eryhtro-*thiolbutyrine¹⁸ and α -amino- β -ethylvaleric acid¹⁹ were prepared in the laboratory. Mixed disulphides were obtained by bubbling oxygen through alkaline solutions of the thiol compounds. S-Carboxymethyl derivatives were prepared by reaction with iodoacetic acid at pH 7.

Penicillins

The penicilloic acid of isopenicillin N was obtained by alkaline degradation of the penicillin²⁰; 6-aminopenicillanic acid (6-APA) was a gift from N.V. Gist-Brocades (Delft, The Netherlands).

Peptides

Reduced and oxidized glutathione were products of Koch-Light Labs. (Colnbrook, Great Britain). The dipeptides L-cystinyl-bis-L-valine and L-cystinyl-bis-Dvaline were prepared by a modification of the procedure of Roeske^{21,22}. The tripeptides bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis^L-valine and bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-D-valine were synthesized as described elsewhere²². The thiol forms of these peptides (LL, LD, LLL and LLD) were obtained by reduction with dithiothreitol (DTT). Bis- δ -(L- α -aminoadipyl)-L-cystine and bis- γ -(L-glutamyl)-L-cystine were prepared by the action of carboxypeptidase A (Koch-Light) on the LLL-tripeptide and oxidized glutathione, respectively; the cysteine forms of these peptides were obtained by reduction with DTT.

Chemicals

All reagents for the preparation of the lithium buffers and the ninhydrin reagent were obtained from E. Merck (Darmstadt, G.F.R.). No filtering of the buffers or of the lithium hydroxide solution was necessary; the former were stored at 4°. Ninhydrin reagent was prepared as described in the Technicon manual²³. Redistilled deionized water was used throughout.

Chromatographic conditions

The equipment consisted of a Technicon amino acid analyzer with a column $(140 \times 0.6 \text{ cm})$ filled with Chromobeads Type B (a strongly acidic cation exchange resin) in the lithium form; two batches of this resin (designated as resin I and resin II) were tested. The solutions used in the Autograd are shown in Table I.

TABLE I

Chamber No.	Buffer 1 (pH 2.75), ml	Buffer 2 (pH 3.01), ml	Buffer 3 (pH 6.50), ml
1	98*		
2	50	50	
3, 4, 5, 6		100	
7, 8, 9	-	-	100

GRADIENT FOR THE NINE-CHAMBERED AUTOGRAD

* Plus 2 ml of isopropyl alcohol.

Buffers of pH 3.01 and pH 6.50 were prepared as described by Vega and Nunn⁵; buffer of pH 2.75 was obtained by acidification (with 6 M hydrochloric acid) of the buffer of pH 3.01.

Samples were loaded on the column in 0.20 M lithium citrate buffer of pH 2.20; in the presence of thiol compounds, 5–10 mg of DTT were added. Elution was carried out at 37° with a flow-rate of 32 ml/h for 7.45 h, and at 55° with a flow-rate of 40 ml/h for the remainder of the chromatogram (back-pressure 400 p.s.i.). To accelerate elution of arginine, 100 ml of buffer of pH 6.50 was added to chamber No. 9 after 22.15 h. At the completion of each run, the column was washed with 0.3 M LiOH for 2 h at 70° and regenerated at the same temperature with buffer of pH 2.75 for 1 h.

RESULTS AND DISCUSSION

Since this work was originally intended for the separation of precursors of penicillin, which are mostly acidic peptides containing α -aminoadipic acid, the elution

NOTES

system was adapted for optimal resolution in the first part of the chromatogram. The gradient of Vega and Nunn⁵ was altered in three ways: 100 ml of buffer was placed in each chamber of the Autograd, a third buffer of pH 2.75 was used, and the content of the pH 6.50 buffer was lowered. For the same reason, the temperature was kept at 37° for 7.45 h, then increased to 55°.

The positions of all 145 ninhydrin-positive compounds on the chromatogram of resin I are shown in Fig. 1; the numbers associated with the various peaks refer to the compounds listed in Table II. As compared with the system of Vega and Nunn⁵, the elution orders of cystine and valine, of phenylalanine and β -alanine and of the cystathionines and methionine are reversed; also, the total analysis time up to arginine is lengthened to over 25 h.

A number of compounds not tested by Vega and Nunn⁵ were well separated. Allo- γ -hydroxyglutamic acid was eluted before allo- β -hydroxyglutamic acid. The three amino sugars tested (glucosamine, mannosamine and galactosamine) were well resolved. In addition, good separations were obtained for most of the diastereo-

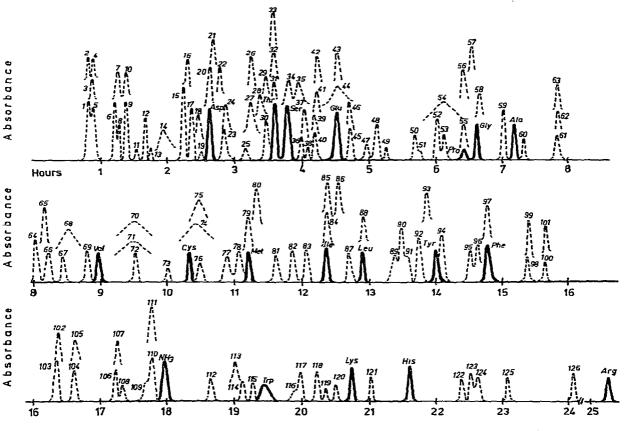


Fig. 1. Elution positions of 145 ninhydrin-positive compounds on resin I. The 18 physiological amino acids, and ammonia, are indicated by solid lines. For identification of numbered peaks (broken lines) see Table II.

:

TABLE II

.

.

IDENTIFICATION OF PEAKS IN FIG. 1

1Cysteic acid50Cysteine2Homocysteic acid51three-Thiolbutyrine3Cysteinesulphinic acid52S-Methylysteine4O-Phosphostrine53 a -Aminoadipic acid5O-Phosphoserine54Glutathione (oxidized)6Taurine55 a -throwyleucine7Penicillaminic acid56S-Carboxymethylmoncysteine8three- β -Hydroxyaspartic acid57 β -Hydroxyleucine9Phosphoethanolarnine58Penicillamine (reduced)10Levuline acid51Isoserine11Dithiothreitol60Lanthionine (peak 1)12 a -thro- β -Hydroxyaspartic acid61Citrulline13Hoz-hydroxyglutathica61Glucosamine14S-Carboxymethylgutathica63S-Ethylzysteine15Allo- β -hydroxyglutamic acid66S-Ethylzysteine16S-Methylcysteine sulphoxides65S-Ethylzysteine17Allo- β -hydroxyglutamic acid66Bis- γ -(α -aminoadipyl)-t-cystine18S-Carboxymethylcysteine69Galactosamine193-Hydroxyprolic71Bis- δ -(α -aminoadipyl)-t-cystinyl-bis-t-valine20S-Carboxymethylgutathione72Fipecolic acid21S-Methylgutathione73Fipecolic acid22S-Methylgutathione74Bis- δ -($-\alpha$ -aminoadipyl)-t-cystinyl-bis-t-valine23Glutathione (reduced)756-Aminopenicillania acid	Peak No.	Compound	Peak No.	Compound
2Homocysteic acid51three-Thiolbutyrine3Cysteinesulphinic acid52S-Methyleysteine4O-Phosphoetneonine53 α -Aminoadipic acid5O-Phosphoetneonine54Glutathione (oxidized)7Penicillaminic acid56S-Carboxymethylhomocysteine9Phosphoethanolamine58Penicillamine (reduced)10Levulinic acid51 β -Hydroxyleucine11Dithiothreitol60Lanthionine (peak 1)12erythro- β -Hydroxyaspartic acid61Citrutline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathione63 α -Aminoisobutyric acid15Allo- γ -hydroxyglutamic acid66 α -Aminobutyric acid16S-Methylcysteine sulphoxide65S-Ethylcysteine17Allo- β -hydroxyglutamic acid68Bis- γ -(L- α -aminoadipyl)-L-cystine18Carboxymethylcysteine79Bis- δ -(L- α -aminoadipyl)-L-cystine20S-Carboxymethylcysteine70Bis- δ -(L- α -aminoadipyl)-L-cystine21S-Carboxymethylcysteine71Bis- δ -(L- α -aminoadipyl)-L-cystine22Jaminosuccinic acid (peak 1)71Bis- δ -(L- α -aminoadipyl)-L-cystine23A-Hydroxypoline74Bis- δ -(L- α -aminoadipyl)-L-cystine24S-Methylglutathione79Phencillamine254-Hydroxypoline74Bis- δ -(L- α -aminoadipyl)-L-cystine2614Mica-Aminoadipyl)-L-cystei	1	Cysteic acid	50	Cysteine
3Cysteinesubphinic acid52S-Methylcysteine4O-Phosphotreonine53 a -Aminoadipic acid5O-Phosphoserine54Glutathione (oxidized)6Taurine55 $erythro-Thiolbutyrine7Penciallaminic acid56S-Carboxymethylhomocysteine8threo-\beta-Hydroxyaspartic acid57\beta-Hydroxyloucine9Phosphothanolamine58Penciallamine (reduced)10Levulinic acid50Isoserine11Dithiothreitol60Lanthionine (peak 1)12erythro-\beta-Hydroxyglutamic acid6413Urea63a-Aminoisobutyric acid14S-Carboxymethylgutathica63S-Ethylcysteine15Allo-j-hydroxyglutamic acid64Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-j-hydroxyglutamic acid68a-Aminobutyric acid18S-Carboxymethylpencillamine70Bis-\sqrt{1-a}-aminoadipyl)-L-cystine193-Hydroxypipecolic acid68Bis-\sqrt{1-a}-aminoadipyl)-L-cystine20SCarboxymethylpencillimine73Bis-\sqrt{1-a}-aminoadipyl)-L-cystine21Diaminosuccinic acid (peak 1)71Bis-\sqrt{1-a}-aminoadipyl)-L-cystine23Glutathione74Bis-\sqrt{1-a}-aminoadipyl)-L-cystine24Sydroxypoline74Bis-\sqrt{1-a}-aminoadipyl)-L-cystine254-Hydroxypoline74Homocirculline264-Hydroxypoline74<$	2	Homocysteic acid		
4O-Phosphotreonine53a-Aminoadipic acid5O-Phosphoserine54Glutathione (oxidized)6Taurine55 $erythro-Thiolburytine7Penicillaminic acid56S-Carboxymethylhomocysteine9Phosphoethanolamine58Penicillamine (reduced)10Levulinic acid59Isoserine11Dithiothreitol60Lanthionine (peak 1)12erythro-fi-Hydroxyaspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathicacid64Glucosamine15Allo-\gamma-hydroxyglutamic acid66a-Aminosuburyic acid16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-\beta-hydroxyglutamic acid68Bis-\gamma-(-glutamyl)-L-cystine18Cophalosporin C67Mannosamine193-Hydroxypipecolic acid68Bis-\gamma-(-glutamyl)-L-cystine20S-Carboxymethylepsteine79Bis-\delta-(-a-aminoadipyl)-L-cystine21S-Carboxymethylepsteine71Bis-\delta-(-a-aminoadipyl)-L-cystine22Diaminosuccinic acid (peak 1)71Bis-\delta-(-a-aminoadipyl)-L-cystine23A-Hydroxyproline74Homocitrulline24S-Methylglutathione75Februlylicine254-Hydroxyproline76Homocysteine2610/atine sulphoxide (peak 2)756-Aminopanicillanic acid2674-Hydroxyproline$	3	Cysteinesulphinic acid	52	
5O-Phosphoserine54Glutathione (oxidized)6Taurine55 $erythro-β$ -Hydroxyaspartic acid567Penicillaminic acid56S-Carboxymethylhomocysteine8three-β-Hydroxyaspartic acid57 $β$ -Hydroxyleucine9Phosphoethanolamine58Penicillamine (reduced)10Levulinic acid59Isoserine11Dithiothreitol60Lanthionine (peak 1)12 $erythro-β$ -Hydroxyaspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylgutanic acid63 a -Aminoisobutyric acid15Allo-γ-hydroxyigutamic acid66 a -Aminosine16S-Methyleysteine sulphoxides65S-Ethyleysteine16S-Methyleysteine acid67Mannosamine17Allo-β-hydroxyigutamic acid68Bis-γ-(glutamyl)-L-cystine20S-Carboxymethylepnicillamine70Bis-δ-(L-a-aminoadipyl)-L-cystine21Diaminosuccinic acid (peak 1)71Bis-δ-(L-a-aminoadipyl)-L-cystine23Glutathione (reduced)72 e -Aminoadipyl)-L-cystine24S-Methylgutathione74Bis-δ-(L-a-aminoadipyl)-L-cystine254-Hydroxyatine74Bis-δ-(L-a-aminoadipyl)-L-cystine26Diaminosuccinic acid (peak 2)756-Aminoadipyl)-L-cystine27Penicillanine74Bis-δ-(L-a-aminoadipyl)-L-cystine28Ap-(L-Glutamyl)-L-cysteine77Phenylglycine </td <td></td> <td></td> <td>53</td> <td></td>			53	
6Taurine55 $erythro-Thiobutyrine7Penicillaminic acid55erythro-Thiobutyrine8three-β-Hydroxyaspartic acid57β-Hydroxyleucine9Phosphoethanolamine58Penicillamine (reduced)10Levulinic acid59Isoserine11Dithiothreitol60Lanthionine (peak 1)12erythro-β-Hydroxyaspartic acid61Citrufline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathione63α-Aminoisobutyric acid15Allo-γ-hydroxyglutamic acid66Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-β-hydroxyglutamic acid68Bis-γ-(1-glutamyl)-L-cystine20S-Carboxymethylcysteine69Glactosamine21S-Carboxymethylpencillamine70Bis-δ-(L-α-aminoadipyl)-L-cystine22Diaminosuccinic acid (peak 1)71Bis-δ-(L-α-aminoadipyl)-L-cystine23S-Methylglutathione73Pipecolic acid24S-Methylglutathione74Bis-δ-(L-α-aminoadipyl)-L-cystinyl-bis-D-valine25A-Hydroxyproline76Homocysteine26Methionine sulphoxide (peak 2)76Homocysteine27Penicilloic acid of isopenicillin N76Homocysteine28Allo-threonine77Phenylglycine39Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-peniclamine30A$			54	
7Penicillaminic acid56S-Carboxymethylhomocysteine8threo- β -Hydroxyaspartic acid57 β -Hydroxyleucine9Phosphoethanolamine58Penicillamine (reduced)10Levulinic acid59Isoserine11Dithiothreitol60Lanthionine (peak 1)12erythro- β -Hydroxyaspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathione63 α -Aminoisobutyric acid15Allo- γ -hydroxyglutamic acid66 α -Aminoisobutyric acid16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo- β -hydroxyglecolic acid68Bis- γ -($-c_{glutamyl}$)- $-cystine20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine70Bis-\delta-(-\alpha-aminoadipyl)-1-cystine23Glutathione (reduced)72\alpha-Aminopinelic acid24S-Methylglutathione73Pipecolic acid25A-Hydroxyproline74Bis-\delta-(1-\alpha-aminoadipyl)-1-cystinyl-bis-1-valine26Diaminosuccinic acid (peak 1)76Homocysteine27Penicillinic acid of isopenicillin76Homocysteine28\gamma-(L-Glutamyl)-1-cysteine77Phenylglycine29Methionine sulphoxide (peak 2)80Mixed disulphide of 1-cysteine and 0-peniclamine35\delta-(-\alpha-Aminoadipyl)-1-cysteine75Cystathionine36\delta-(-\alpha-Aminoadi$	6	Taurine	55	
8three- β -Hydroxyaspartic acid57 β -Hydroxyleucine9Phosphoethanolamine58Penicillamine (reduced)11Dithiothreitol60Lanthionine (peak 1)12erythro- β -Hydroxyaspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutamic acid63 α -Aminoisobutyric acid15Allo- p -hydroxyglutamic acid66S-Ethylcysteine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo- β -hydroxyglutamic acid66 α -Aminobutyric acid18S-Carboxymethylgutamic acid68Bis- γ -(L- α -aminoadipyl)-L-cystine193-Hydroxypipecolic acid68Bis- γ -(L- α -aminoadipyl)-L-cystine20S-Carboxymethylgenicillamine70Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-L-valine21S-Carboxymethylgenicillamine71Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-D-valine22S-Methylglutathione73Pipecolic acid23S-Methylglutathione74Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-D-valine24S-Carboxynethylpenicillin N76Homocysteine254-Hydroxyyaline76Homocysteine26y-(t-Glutamyl)-t-cysteine77Phencillanic acid27Penicillonine sulphoxide (peak 1)78Homocitrulline36 δ -(L- α -Aminoadipyl)-L-cysteine78Homocitrulline37 δ -Hydroxyyaline81Allo-cystathionine	7	Penicillaminic acid	56	
9Phosphoethanolamine58Penicillamine (reduced)10Levulinic acid59Isoserine10Levulinic acid50Isoserine11Dithiothreitol60Lanthionine (peak 1)12 $erythro-\beta$ -Hydroxyspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathione63 a -Aminobutyric acid15Allo- β -hydroxyglutamic acid66 a -Aminobutyric acid16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo- β -hydroxyglutamic acid66 a -Aminobutyric acid18S-Carboxymethylcysteine69Galactosamine20S-Carboxymethylpenicillamine70Bis- δ -($-a$ -aminoadipyl)-L-cystine21Diaminosuccinic acid (peak 1)71Bis- δ -($-a$ -aminoadipyl)-L-cystinyl-bis-L-valine23Glutathione (reduced)72 a -Aminopimelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -($-a$ -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28y-(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine	8	threo- β -Hydroxyaspartic acid		
10Levulinic acid59Isoserine11Dithiothreitol60Lanthionine (peak 1)12 $erythro.p-Hydroxyaspartic acid60Lanthionine (peak 1)13Urea61Citrulline14S-Carboxymethylglutamic acid63a-Aminosbutyric acid15Allo-y-hydroxyglutamic acid66a-Aminobutyric acid16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-f-hydroxyglutamic acid66a-Aminobutyric acid18S-Carboxymethylgeutamic acid66a-Aminobutyric acid193-Hydroxypipecolic acid69Galactosamine21S-Carboxymethylgeutamic acid (peak 1)71Bis-\delta-(L-a-aminoadipyl)-L-cystine22Diaminosuccinic acid (peak 1)71Bis-\delta-(L-a-aminoadipyl)-L-cystinyl-bis-L-valine23Althoine sulphoxide (peak 2)756-Aminopenicillanic acid24S-Carboxymethyloxide (peak 2)756-Aminopenicillanic acid254-Hydroxypaline71Homoctrulline26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloine sulphoxide (peak 1)76Homoctrulline28Y-(L-a-Aminoadipyl)-L-cysteine79Norvaline29Methionine sulphoxide (peak 2)76Amino-fb-thydroxybutyric acid37Ø-Methylthreonine7077Phenylgycine38Allo-threonine7174Homoctrulline39Ø-Hydroxypypiceolic acid76<$				
12 $erythro-\beta-Hydroxyaspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathione63a-Aminosiboutyric acid15Allo-y-hydroxyglutamic acid64Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-f-hydroxyglutamic acid66a-Aminosibutyric acid18S-Carboxymethylgetolic acid68Bis-\gamma-(t-glutamyl)-t-cystine193-Hydroxypipecolic acid69Galactosamine20S-Carboxymethylcysteine70Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine21S-Carboxymethylcysteine71Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine22Diaminosuccinic acid (peak 1)71Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine23Glutathione (reduced)72a-Aminospinceillanic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine26Jaminosuccinic acid (peak 2)766-Aminospenicillanic acid27Penicilloic acid of isopenicillin N76Homocrysteine28\gamma-(t-Glutamyl)-t-cysteine77Norvaline39Methionine sulphoxide (peak 2)80Mixed disulphide of t-cysteine and to-penic33\beta-Hydroxyproline81Allo-cystathionine34\delta-(t-a-Aminoadipyl)-t-cysteinyl-t-82Dienkolic acid39Muramic acid83<$				
12 $erythro-\beta-Hydroxyaspartic acid61Citrulline13Urea62Lanthionine (peak 2)14S-Carboxymethylglutathione63a-Aminosiboutyric acid15Allo-y-hydroxyglutamic acid64Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-f-hydroxyglutamic acid66a-Aminosibutyric acid18S-Carboxymethylgetolic acid68Bis-\gamma-(t-glutamyl)-t-cystine193-Hydroxypipecolic acid69Galactosamine20S-Carboxymethylcysteine70Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine21S-Carboxymethylcysteine71Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine22Diaminosuccinic acid (peak 1)71Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine23Glutathione (reduced)72a-Aminospinceillanic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis-\delta-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine26Jaminosuccinic acid (peak 2)766-Aminospenicillanic acid27Penicilloic acid of isopenicillin N76Homocrysteine28\gamma-(t-Glutamyl)-t-cysteine77Norvaline39Methionine sulphoxide (peak 2)80Mixed disulphide of t-cysteine and to-penic33\beta-Hydroxyproline81Allo-cystathionine34\delta-(t-a-Aminoadipyl)-t-cysteinyl-t-82Dienkolic acid39Muramic acid83<$	11	Dithiothreitol	60	Lanthionine (peak 1)
13Urea62Lanthionine (peak 2)14S-Carboxymethylgutamic acid63 a -Aminoisobutyric acid15Allo-y-hydroxyglutamic acid64Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-β-hydroxyglutamic acid66 a -Aminobutyric acid18Cephalosporin C67Mannosamine193-Hydroxyplipecolic acid68Bis-y-(t-glutamyl)-t-cystine20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine70Bis-δ-(t-a-aminoadipyl)-t-cystine22Diaminosuccinic acid (peak 1)71Bis-δ-(t-a-aminoadipyl)-t-cystinyl-bis-t-valine23Glutathione (reduced)72 a -Aminopimelic acid24S-Methylglutathione73Pipecolic acid25A-Hydroxyproline74Bis-δ-(t-a-aminoadipyl)-t-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28y-(t-Glutamyl)-t-cysteine79Norvaline29Methionine sulphoxide (peak 1)71Homocysteine31Methionine sulphoxide (peak 2)80Mixed disulphide of t-cysteine and D-penic lamine33 β -Hydroxyvaline81Allo-stoleucine34 δ -(t-a-Aminoadipyl)-t-cysteine82Ethionine35 δ -(t-a-Aminoadipyl)-t-cysteine83Djenkolic acid36Allo-4-hydroxyproline </td <td>12</td> <td>erythro-B-Hydroxyaspartic acid</td> <td></td> <td></td>	12	erythro-B-Hydroxyaspartic acid		
14S-Carboxymethylglutathione63 a -Aminoisobutyric acid15Allo- γ -hydroxyglutamic acid64Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo- β -hydroxyglutamic acid66 a -Aminobutyric acid18Cephalosporin C67Mannosamine20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine69Galactosamine22Diaminosuccinic acid (peak 1)71Bis- δ -(t- a -aminoadipyl)-t-cystinyl-bis-t-valine23Glutathione (reduced)72 a -Aminopimelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(t- a -aminoadipyl)-t-cystinyl-bis-t-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)70Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of t-cysteine and to-penic lamine33 β -Hydroxyproline81Allo-soleucine34 δ -(t- a -Aminoadipyl)-t-cysteinyl-t- valine81Allo-cystathionine35 δ -(t- a -Aminoadipyl)-t-cysteinyl-t- valine83Djenkolic acid36Allo-4-hydroxyproline87 a -Amino- β -hydroxybutyric acid35 δ -(t- a -Aminoadipyl)-t-cysteinyl-t- valine <td></td> <td></td> <td></td> <td></td>				
15Allo- γ -hydroxyglutamic acid64Glucosamine16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo- β -hydroxyglutamic acid66 α -Aminobutyric acid18Cephalosporin C67Mannosamine193-Hydroxypipecolic acid68Bis- γ -(1-glutamyl)-L-cystine10S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine69Galactosamine22Diaminosuccinic acid (peak 1)71Bis- δ -(L- α -aminoadipyl)-L-cystingl-bis-L-valine23Glutathione (reduced)72 α -Aminopanielic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(L- α -aminoadipyl)-L-cystingl-bis-D-valine26Diaminosuccinic acid (peak 2)766-Aminopencillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 2)78Mixed disulphide of L-cysteine and D-penic lamine31 β -Hydroxyvaline81Allo-sioleucine32 $All-d-threonine$ 82Ethionine33 ϕ -L- α -Aminoadipyl)-L-cysteinyl-L- valine8134 ϕ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine8235O-Methylserine8136Allo-4hydroxyproline8737O-Methylserine8138Allo-4hydroxyptoclic acid39 <td< td=""><td></td><td></td><td></td><td></td></td<>				
16S-Methylcysteine sulphoxides65S-Ethylcysteine17Allo-β-hydroxyglutamic acid66 a -Aminobutyric acid18Cephalosporin C67Mannosamine193-Hydroxypipecolic acid68Bis-γ-(t-glutamyl)-L-cystine20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylepenicillamine70Bis-δ-(t-a-aminoadipyl)-L-cystine22Diaminosuccinic acid (peak 1)71Bis-δ-(t-a-aminoadipyl)-L-cystinyl-bis-L-valine23Glutathione (reduced)72 a -Aminopintelic acid24S-Methylgutathione73Pipecolic acid254-Hydroxyproline74Bis-δ-(t-a-aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28γ-(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine33β-Hydroxyaline81Allo-cystathionine34δ-(L-a-Aminoadipyl)-L-cysteine82Ethionine35δ-(L-a-Aminoadipyl)-L-cysteine82Ethionine36Allo-thydroxyproline83Allo-cystathionine37O-Methylsterine84Allo-cystathionine38Allo-4-hydroxyproline </td <td></td> <td></td> <td></td> <td></td>				
17Allo- β -hydroxyglutamic acid66 a -Aminobutyric acid18Cephalosporin C67Mannosamine193-Hydroxypipecolic acid68Bis- γ -(ι - a -lutamyl)-L-cystine20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine69Galactosamine22Diaminosuccinic acid (peak 1)71Bis- δ -(t - a -aminoadipyl)-L-cystine23Glutathione (reduced)72 a -Aminopimelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(t - a -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocrulline30Methionine sulphoxide (peak 2)75Allo-threonine31 β -Hydroxyproline81Allo-cysteine and D-penic lamine35 δ -(t - a -Aminoadipyl)-L-cysteine82Ethionine36O-Methylserine83Cystathionine37O-Methylserine87 a -Amino- β -hydroxyputyric acid38Allo-4-hydroxyproline87 a -Amino- β -hydroxyputyric acid38Allo-4-hydroxypoline87 a -Amino- β -hydroxyputyric acid38Allo-4-hydroxypoline87 a -Amino- β -hydroxyputyric acid39Muramic acid				
18Cephalosporin C67Mannosamine193-Hydroxypipecolic acid68Bis- γ -(L -glutamyl)-L-cystine20S-Carboxymethylysteine69Galactosamine21S-Carboxymethylpenicillamine70Bis- δ -(L - a -aminoadipyl)-L-cystine22Diaminosuccinic acid (peak 1)71Bis- δ -(L - a -aminoadipyl)-L-cystine23Glutathione (reduced)72 a -Aminopinelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(L - a -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 1)78Homocitrulline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine32Allo-threonine81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L - a -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid35 δ_1 -(a -Aminoadipyl)-L-cysteinyl-L- valine83Allo-soleucine36Allo-thydroxyproline81Allo-isoleucine37O-Methylkeronine82Cystathionine38Allo-4-hydroxyproline87 a -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine </td <td></td> <td></td> <td></td> <td></td>				
193-Hydroxypipecolic acid68Bis-y-(L-glutamyl)-L-cystine20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine69Galactosamine21Diaminosuccinic acid (peak 1)71Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-L-valine23Glutathione (reduced)72 α -Aminopimetic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28y-(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphone79Norvaline31Methionine sulphoxide (peak 1)78Homocysteine32 β -Hydroxyvaline81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxybutyric acid40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 α_e -Chiaminop/melic acid42S-Carbamylcysteine91Norleucine43 δ -(L- α -Ami				•
20S-Carboxymethylcysteine69Galactosamine21S-Carboxymethylcysteine70Bis- δ -(ι - α -aminoadipyl)- ι -cystine22Diaminosuccinic acid (peak 1)71Bis- δ -(ι - α -aminoadipyl)- ι -cystinyl-bis- ι -valine23Glutathione (reduced)72 α -Aminopimelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxypoline74Bis- δ -(ι - α -aminoadipyl)- ι -cystinyl-bis- ι -cystinyl-bis-dy-cystine29Methionine sulphoxide (peak 2)756-Aminocymuline31Methionine sulphoxide (peak 2)80Mixed disulphide of ι -cysteine and ι -cysteine33				
21S-Carboxymethylpenicillamine70Bis- δ -(L- a -aminoadipyl)-L-cystine22Diaminosuccinic acid (peak 1)71Bis- δ -(L- a -aminoadipyl)-L-cystinyl-bis-L-valine23Glutathione (reduced)72 a -Aminopimelic acid24S-Methylglutathione72 a -Aminopimelic acid254-Hydroxyproline74Bis- δ -(L- a -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine29Methionine sulphoxide (peak 1)78Homocysteine20Methionine sulphone79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic36 β -Hydroxyvaline81Allo-isoleucine37 ϕ -L(- a -Aminoadipyl)-L-cysteinyl-L-82Ethionine36 ϕ -L(- a -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid37 O -Methyltreonine85Cystathionine38Allo-4-hydroxyproline87 a -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphutyric acid39Muramic acid81Soglutarmine41Allo-4-hydroxypipecolic acid90 a_e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- a -Aminoadipyl)-L-cysteinyl-D-93 a -Amino- β -ethylvaleric acid47<				
22Diaminosuccinic acid (peak 1)71Bis- δ -(L- a -aminoadipyl)-L-cystinyl-bis-L-value23Glutathione (reduced)72 a -Aminopinelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(L- a -aminoadipyl)-L-cystinyl-bis-D-value26Diaminosuccinic acid (peak 2)766-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- a -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid35 δ -(L- a -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid36O-Methylserine86Allo-cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 a -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxybenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a_e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- a -Aminoadi				
23Glutathione (reduced)72 a -Aminoppinelic acid24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(L- a -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic31Methionine sulphoxide (peak 2)81Allo-isoleucine31Methionine sulphoxide (peak 2)81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- a -Aminoadipyl)-L-cysteinyl-L- valine81Allo-isoleucine35 δ -(L- a -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine82Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxypoline87 a -Amino- β -hydroxybutyric acid39Muramic acid88 3 -4-Dihydroxybutynic acid40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a - ϵ -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline91Cycloserine44 δ -(L- a -Aminoadipyl)-L-cysteinyl-D- valine			-	
24S-Methylglutathione73Pipecolic acid254-Hydroxyproline74Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic32Allo-threonine81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine81Allo-isoleucine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine81Allo-cystathionine36O-Methyltreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxybenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypteolic acid90 α_c -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-valine93 α -Amino- β -ethylvaleric acid45Glutamine95 β -Alanine46Homoserine95 β -Alanin	23	Glutathione (reduced)		
254-Hydroxyproline74Bis- δ -(L- α -aminoadipyl)-L-cystinyl-bis-D-valine26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine81Allo-isoleucine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine81Allo-sioleucine36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 α_s -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homo cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96 </td <td></td> <td></td> <td></td> <td>•</td>				•
26Diaminosuccinic acid (peak 2)756-Aminopenicillanic acid27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxide (peak 2)79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic32Allo-threonine81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino $-\beta$ -hydroxybutyric acid39Muramic acid88 $3,4$ -Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,ϵ -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino $-\beta$ -ethylvaleric acid45Glutamine95 β -Alanine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of pL-homocysteine and pL-hom cysteine48Sar				
27Penicilloic acid of isopenicillin N76Homocysteine28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphoxe79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic32Allo-threeonine81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 α,ε -Diaminopimelic acid42 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homocysteine and DL-homocysteine and DL-homocysteine and DL-homocysteine and D- penicillamine45Glutamine95 β -Alanine46Homoserine95 β -Alanine <td< td=""><td></td><td></td><td></td><td></td></td<>				
28 γ -(L-Glutamyl)-L-cysteine77Phenylglycine29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphone79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic32Allo-threonine1Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 α_c -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-valine93 α -Amino- β -ethylvaleric acid45Glutamine95 β -Alanine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D-penicillamine48Sarcosine96Mixed disulphide of DL-homocysteine and D-penicillamine				-
29Methionine sulphoxide (peak 1)78Homocitrulline30Methionine sulphone79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine32Allo-threonine81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,ε -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homa cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine				•
30Methionine sulphone79Norvaline31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine32Allo-threonine81Allo-isoleucine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a_e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-home cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine48Sarcosine95 β -Alanine				
31Methionine sulphoxide (peak 2)80Mixed disulphide of L-cysteine and D-penic lamine32Allo-threonine1Allo-isoleucine133 β -Hydroxyvaline81Allo-isoleucine8134 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a_e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homo- cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine48Sarcosine95 β -Alanine				
32Allo-threoninelamine33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 α,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homo-46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D-48Sarcosine91Mixed disulphide of DL-homocysteine and D-				
33 β -Hydroxyvaline81Allo-isoleucine34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L-83Djenkolic acidvaline84Penicillamine (oxidized)36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 α,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homo- cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine		• •	00	
34 δ -(L- α -Aminoadipyl)-L-cysteine82Ethionine35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homo- cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine			81	
35 δ -(L- α -Aminoadipyl)-L-cysteinyl-L- valine83Djenkolic acid36O-Methylthreonine84Penicillamine (oxidized)36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D- valine93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-home cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine				
valine84Penicillamine (oxidized)36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid88 $3,4$ -Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-home46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D-48Sarcosine96Mixed disulphide of DL-homocysteine and D-				
36O-Methylthreonine85Cystathionine37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid88 $3,4$ -Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-home46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D-48Sarcosine96Mixed disulphide of DL-homocysteine and D-	55			
37O-Methylserine86Allo-cystathionine38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid88 $3,4$ -Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-homo- cysteine46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine	36			
38Allo-4-hydroxyproline87 α -Amino- β -hydroxybutyric acid39Muramic acid88 $3,4$ -Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-home46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D-48Sarcosine96Mixed disulphide of DL-homocysteine and D-		=		
39Muramic acid883,4-Dihydroxyphenylalanine40Asparagine89Isoglutamine41Allo-4-hydroxypipecolic acid90 a,e -Diaminopimelic acid42S-Carbamylcysteine91Norleucine43 β -Methoxyvaline92Cycloserine44 δ -(L- α -Aminoadipyl)-L-cysteinyl-D-93 α -Amino- β -ethylvaleric acid45Glutamine94Mixed disulphide of L-cysteine and DL-home46Homoserine95 β -Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D-48Sarcosine96Mixed disulphide of DL-homocysteine and D-				
 40 Asparagine 41 Allo-4-hydroxypipecolic acid 42 S-Carbamylcysteine 43 β-Methoxyvaline 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 49 Isoglutamine 49 a,e-Diaminopimelic acid 40 a,e-Diaminopimelic acid 40 a,e-Diaminopimelic acid 40 a,e-Diaminopimelic acid 41 Norleucine 42 Cycloserine 43 β-Methoxyvaline 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 49 Isoglutamine 49 a,e-Diaminopimelic acid 40 a,e-Diaminopimelic acid 41 Norleucine 42 Cycloserine 43 α-Amino-β-ethylvaleric acid 44 Mixed disulphide of L-cysteine and DL-homocysteine and D-penicillamine 				
 41 Allo-4-hydroxypipecolic acid 42 S-Carbamylcysteine 43 β-Methoxyvaline 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 41 Allo-4-hydroxypipecolic acid 42 a,e-Diaminopimelic acid 49 a,e-Diaminopimelic acid 41 Norleucine 42 Cycloserine 43 α-Amino-β-ethylvaleric acid 44 Mixed disulphide of L-cysteine and DL-homocysteine 45 β-Methoxyvaline 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 49 α,e-Diaminopimelic acid 40 α,e-Diaminopimelic acid 41 Norleucine 42 Cycloserine 43 α-Amino-β-ethylvaleric acid 44 Δ-(L-α-Amino-β-ethylvaleric acid 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 				
 42 S-Carbamylcysteine 43 β-Methoxyvaline 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 91 Norleucine 92 Cycloserine 93 α-Amino-β-ethylvaleric acid 94 Mixed disulphide of L-cysteine and DL-homocysteine 95 β-Alanine 96 Mixed disulphide of DL-homocysteine and D-penicillamine 				
 43 β-Methoxyvaline 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 43 β-Methoxyvaline 44 92 Cycloserine 93 α-Amino-β-ethylvaleric acid 94 Mixed disulphide of L-cysteine and DL-homocysteine 95 β-Alanine 96 Mixed disulphide of DL-homocysteine and D-penicillamine 				
 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 44 δ-(L-α-Aminoadipyl)-L-cysteinyl-D-yaline 45 Glutamine 46 Homoserine 47 4-Oxopipecolic acid 48 Sarcosine 49 A-Amino-β-ethylvaleric acid 49 Mixed disulphide of L-cysteine and DL-homocysteine and DL-homocysteine and D-penicillamine 				
valine94Mixed disulphide of L-cysteine and DL-home45Glutaminecysteine46Homoserine95474-Oxopipecolic acid9648Sarcosinepenicillamine				
45Glutaminecysteine46Homoserine95β-Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine48Sarcosinepenicillamine	-1-1			
46Homoserine95β-Alanine474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine48Sarcosinepenicillamine	15		74	
474-Oxopipecolic acid96Mixed disulphide of DL-homocysteine and D- penicillamine48Sarcosine96			05	
48 Sarcosine penicillamine				
			סצ	
47 J-rryaroxypipeconc acia 91 U-Benzyiserine			07	
	49	acia	91	

TABLE II (continued)

Peak No.	Compound	Peak No.	Compound
98	β -Aminoisobutyric acid	112	δ -Aminovaleric acid
99	δ -Aminolevulinic acid	113	Valinol
100	L-Cysteinyl-L-valine	114	5-Hydroxylysine
101	L-Cysteinyl-D-valine	115	Allo-5-hydroxylysine
102	Argininosuccinic acid	116	Creatinine
103	Homocystine	117	α,γ -Diaminobutyric acid
104	2-Aminobutyric acid	118	Ornithine
105	S-Benzylcysteine	119	Valinamide
106	5-Hydroxytryptophan	120	ε-Aminocaproic acid
107	a-Aminocaprylic acid	121	1-Methylhistidine
108	Ethanolamine	122	3-Methylhistidine
109	Kynurenine	123	Carnosine
110	L-Cystinyl-bis-L-valine	124	Homocarnosine
111	L-Cystinyl-bis-D-valine	125	α -Amino- β -guanidinopropionic acid
	2 -	126	Homocysteine thiolactone

isomers of amino acids and peptides tested: e.g., threo- and erythro- β -hydroxyaspartic acid; hydroxyproline, isoleucine, 5-hydroxylysine and their allo-forms; threo- and erythro-thiolbutyrine and the LLL- and LLD-isomers of oxidized and reduced δ -(α aminoadipyl)-cysteinylvaline. However, some of the compounds were not resolved in our system. The strongly acidic amino acids (cysteic acid, homocysteic acid, phosphoserine and phosphothreonine) always eluted with the front. Identical elution times were obtained for threonine and allo-threonine, for aspartic acid and S-carboxymethylcysteine, for the second peak of lanthionine and α -aminoisobutyric acid, for methionine and norvaline, for 3,4-dihydroxyphenylalanine and leucine, and for β -aminoisobutyric acid and δ -aminolevulinic acid. The LL- and LD-epimers of cysteinylvaline and of cystinyl-bis-valine always ran together.

The elution pattern with resin II differed from that with resin I in several respects. Reduced glutathione was eluted after aspartic acid on resin I, but the two were only marginally separated on resin II. Methionine sulphone and threonine were only separated with resin I. The resolution of δ -(L- α -aminoadipyl)-L-cysteine and the LLL-thiol tripeptide was better with resin I than with resin II. Glutamic acid and glutamine were well resolved on resin I, but had the same retention time on resin II. In contrast, the LLD-thiol peptide and glutamic acid were only separated on resin II. Also, separations of cystine and 6-APA, allo-isoleucine and ethionine, and 3-methylhistidine and carnosine were better on resin I, whereas those of citrulline and lanthionine (peak 2), galactosamine and valine, homocitrulline and methionine, a,e-diaminopimelic acid and norleucine, and O-benzylserine and phenylalanine were better on resin II. The differences between the two resins remained constant, although both columns were re-packed several times. Differences in separation on the two resins can be advantageous for the identification of unknown compounds; in this way, glutathione and the LLD-thiol tripeptide (labelled with sulphur-35) could be detected in cultures of *Penicillium chrvsogenum*¹.

ACKNOWLEDGEMENT

٠.

One of us (B.M.) is a fellow of the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw, I.W.O.N.L.

REFERENCES

- 1 P. Adriaens, B. Meesschaert, W. Wuyts, H. Vanderhaeghe and H. Eyssen, Antimicrob. Agents Chemother, 8 (1975) 638.
- 2 J. V. Benson, Jr., M. J. Gordon and J. A. Patterson, Anal. Biochem., 18 (1967) 228.
- 3 J. H. Peters, B. J. Berridge, Jr., J. G. Cummings and S. C. Lin, Anal. Biochem., 23 (1968) 459.
- 4 T. L. Perry, D. Stedman and S. Hansen, J. Chromatogr., 38 (1968) 460.
- 5 A. Vega and P. B. Nunn, Anal. Biochem., 32 (1969) 446.
- 6 G. E. Atkin and W. Ferdinand, Anal. Biochem., 38 (1970) 313.
- 7 C.-P. Kedenburg, Anal. Biochem., 40 (1971) 35.
- 8 Y. Houpert, P. Tarallo and G. Siest, J. Chromatogr., 115 (1975) 33.
- 9 L. Bowie, J. C. Crawhall, N. Gochman, K. Johnson and J. A. Schneider, Clin. Chim. Acta, 68 (1976) 349.
- 10 R. M. Zacharius and E. A. Talley, Anal. Chem., 34 (1962) 1551.
- 11 P. B. Hamilton, Anal. Chem., 35 (1963) 2055.
- 12 Y. Mardens, M. van Sande and J. Caers, Anal. Lett., 4 (1971) 285.
- 13 T. J. McCord and C. G. Skinner, Biochem. Prep., 10 (1963) 18.
- 14 M. Claesen, A. Vlietinck and H. Vanderhaeghe, Bull. Soc. Chim. Belges, 77 (1968) 587.
- 15 K. Rüfenacht, Helv. Chim. Acta, 35 (1952) 762.
- 16 S. Tatsuoka, T. Ueno and K. Hirata, J. Pharm. Soc. Japan, 70 (1950) 229.
- 17 H. Vanderhaeghe and G. Parmentier, J. Amer. Chem. Soc., 82 (1960) 4414.
- 18 J. Hoogmartens, P. J. Claes and H. Vanderhaeghe, J. Org. Chem., 39 (1974) 425.
- 19 L. Antoine, Thesis, University of Leuven, Leuven, 1974.
- 20 H. Vanderhaeghe, A. Vlietinck, M. Claesen and G. Parmentier, J. Antibiot., 27 (1974) 169.
- 21 R. W. Roeske, Biochem. Prep., 10 (1963) 43.
- 22 H. Vanderhaeghe and P. Adriaens, J. Labelled Compounds Radiopharm., 12 (1976) 381.
- 23 Technicon Instruments Co. Ltd., Instruction Manual AAA-1, Technicon AutoAnalyzer, Tarrytown, N.Y., U.S.A.