

On the Quantitative Determination of the Amino Acids and γ -Glutamylpeptides of Onion

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A number of new primary plant substances, mostly amino acids and γ -glutamylpeptides, have been isolated in this laboratory from onion and other plants. The content of these substances in the plants was in most cases roughly calculated on the basis of the yields obtained. Due to the losses taking place during various stages of isolation the results obtained in this way are only

Table 1. Results obtained with onion species using an amino acid analyzer. Contents are given in μg amino acid or peptide/g onion.

Compound	Onion 2321		
	Ethanol extract	Neutral and basic	Acid
γ -Glu-LP	—	—	1965
S-MeCys-sulphoxide	—	347	—
S-(2-COOH-pr)-GSH	523	—	345
LP	1927	1922	—
γ -Glu-S-MeCys	190	—	159
Asp	131	6.5	127
Asn	391	407	1.8
Thr	154	165	+
Ser	166	178	1.4
γ -Glu-Met	127	—	103
Glu	346	10.1	360
Cit	53.0	52.9	—
Pro	28.3	31.4	—
S-MeCys	16.7	15.8	—
Gly	—	14.3	—
Ala	61.3	66.9	—
Cystine + (Abu)	—	3.6	—
γ -Glu-Phe	568	—	418
Val	65.4	59.2	—
Pipecolic acid	+	8.3	—
Met	4.7	4.2	—
S-(Prop-1-enyl)-Cys	9.2	7.8	5.1
Ile	25.3	24.1	—
Leu	78.7	82.6	—
Tyr	162	168	24.6
β -Ala	+	4.4	—
Phe	88.8	93.7	—
γ -Abu	45.4	50.6	—
Ethanolamine	+	4.3	—
Lys	105	109	—
His	116	118	—
Trp	+	—	83.9
Arg	1442	1465	15.0

Abbreviations: LP = S-(prop-1-enyl) cysteine sulfoxide, S-MeCys-sulfoxide = S-methylcysteine sulfoxide, S-(2-COOH-pr)-GSH = S-(2-Carboxypropyl)-glutathione. The others are general abbreviations.

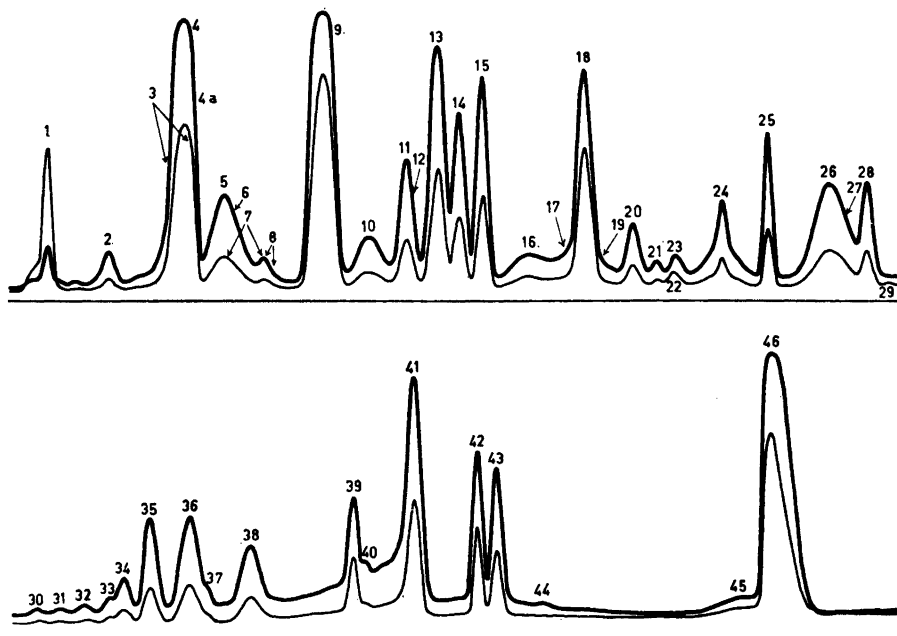


Fig. 1. A chromatogram made of the ethanol extract of onion 2321 with an amino acid analyzer.

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|---|--------------------------------------|
| 1. Sugars | 23. S-Methyleysteine |
| 2. Unknown | 24. Glycine and unknown |
| 3. Isomers of S-methyl-L-cysteine sulphoxide | 25. Alanine |
| 4. γ -Glutamyl-S-(prop-1-enyl)-cysteine sulphoxide | 26. γ -Glutamyl-phenylalanine |
| 4a. Unknown | 27. S-Allylcysteine |
| 5. S-(2-Carboxypropyl)-glutathione | 28. Valine |
| 6. Cycloalliin | 29. Pipecolic acid |
| 7. Isomers of S-propyl-L-cysteine sulphoxide | 30. Methionine |
| 8. Isomers of L-methionine sulphoxide | 31. S-(Prop-1-enyl)-cysteine |
| 9. S-(Prop-1-enyl)-L-cysteine sulphoxide | 32. * |
| 10. γ -Glutamyl-S-methyl cysteine | 33. Unknown |
| 11. Aspartic acid | 34. Isoleucine |
| 12. L-Glutamyl-valine | 35. Leucine |
| 13. Asparagine | 36. Tyrosine |
| 14. Threonine | 37. β -Alanine |
| 15. Serine | 38. Phenylalanine |
| 16. γ -Glutamyl-methionine | 39. γ -Aminobutyric acid |
| 17. γ -Glutamyl-isoleucine | 40. Ethanolamine |
| 18. Glutamic acid | 41. Ammonia |
| 19. S-(2-Carboxy-propyl)-L-cysteine | 42. Lysine |
| 20. Citrulline | 43. Histidine |
| 21. Unknown | 44. Unknown |
| 22. Proline | 45. Tryptophan |
| | 46. Arginine |

approximate. In many cases, as for instance when various onion varieties are compared with one another, quantitative

results are, however, needed. Therefore during the last few years we have used an automatic amino acid analyzer to study

the composition of onion species. The determinations have been made both directly on the ethanol extract of onion and on the neutral, basic, and acid amino acid fractions. The methods used for the separation will be described in a more detailed paper elsewhere.

As control compounds, partly preparations isolated in this laboratory from onion bulb, chive and garlic, partly synthesized in this laboratory (racemic mixtures) and partly Beckman type 1 calibration mixture were used. As examples of our results obtained with some onion varieties Table 1 and Fig. 1 are added to this preliminary report.

Both qualitative and quantitative differences occur in the onion varieties studied in regard to amino acids and peptides. At this stage it cannot yet be said to what extent the differences are due to the variety and to what extent due to the growth circumstances, for example differences in the sulfur content of the soil.

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Acid-Catalyzed Rearrangement of 3-Phenyl-2-bromothiophene

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It has been shown that different isomer distributions are obtained on bromination of 3-phenylthiophene (I) ¹ with various brominating agents. While bromination with *N*-bromosuccinimide (NBS) in carbon tetrachloride led to the expected 2-bromo-3-phenylthiophene (II), bromination with bromine in refluxing acetic acid yielded 5-bromo-3-phenylthiophene (III) and II in a 2:1 ratio. Further experiments have shown that bromination of I with NBS in acetic acid also yields a mixture of II and III and

that I with bromine in CCl₄ yields almost exclusively II. It is thus obvious that the explanation given in Ref. 1 must be incorrect.

A more detailed study of the bromination of I with bromine in acetic acid indicated that II was first formed, but then rearrangement to a mixture of I, II, III, and 2,5-dibromo-3-phenylthiophene (IV) occurred (*cf.* Table 1). Approximately the same mixture was obtained when pure II was refluxed with HBr in acetic acid for 4 h or when III was treated in the same way for 25 h (*cf.* Table 1). After refluxing IV for 25 h, small amounts of II and III could be detected. It thus appears that the bromination of 3-phenylthiophene is reversible and that the ultimately formed product mixture is thermodynamically controlled.

The behaviour of the phenylbromothiophenes thus appears to be more similar to that of halogenopyrroles than to that of halobenzenes. In the pyrrole series protodehalogenation and other types of dehalogenation have been observed with both iodo- and bromopyrroles,²⁻⁵ which therefore are considered to contain "positive" halogen. There is certainly also a connection between the facile halogen-metal exchange in bromothiophenes⁶ and the protodehalogenation of 2-bromo-3-phenylthiophene. Similar results to those described above have also been obtained by Wynberg *et al.*⁷

Experimental. 2-Bromo-3-phenylthiophene was prepared as described in Ref. 1. Column chromatography on alumina (Fluka 507 c, neutral, activity grade 1) with hexane as eluent yielded the analytically pure compound, b.p. 106°C/0.3 mm Hg, $n_D^{20} = 1.6540$. (Found: C 50.0; H 2.91; S 13.4; Br 33.5. Mol.wt. 239. Calc. for C₁₀H₇BrS (239.1): C 50.23; H 2.95; S 13.41; Br 33.41).

5-Bromo-3-phenylthiophene was prepared as described in Ref. 1. Recrystallization from aqueous ethanol yielded the analytically pure compound, m.p. 79–80°C. (Found: C 50.6; H 2.89; S 13.5; Br 33.2. Mol.wt. 239. Calc. for C₁₀H₇BrS (239.1): C 50.23; H 2.95; S 13.41; Br 33.41).

2,5-Dibromo-3-phenylthiophene. 2.02 g (0.0125 mole) of bromine in 20 ml of acetic acid was added dropwise to 1.00 g (0.00625 mole) of 3-phenylthiophene in 20 ml of acetic acid at reflux temperature. After refluxing for 6 h, the mixture was cooled and poured onto ice which caused the precipitation of a brown oil. The mixture was neutralized with 2.5 N