

Short Communications

New α -Keto Acids in Green Plants. α -Ketopimelic Acid, γ -Hydroxy- α -ketopimelic Acid, and Hydroxypyruvic Acid in *Asplenium septentrionale*, α -Keto adipic Acid in Germinating Pea Seeds

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When investigating the mode of formation of the new amino acids α -aminopimelic acid and γ -hydroxy- α -aminopimelic acid which were recently found in this laboratory ^{1,2} in *Asplenium septentrionale*, we have given attention to the keto acids in this plant. Virtanen *et al.*³ have identified keto acids in the form of their 2,4-dinitrophenylhydrazones by means of paper chromatography, and using this method they could confirm the earlier findings in this laboratory with other methods⁴ of the presence of pyruvic acid, oxalacetic acid, and α -ketoglutaric acid in pea plants. If, however, a still greater number of keto acids is to be found simultaneously in the investigated material the separating capacity of this method is not great enough, especially as the hydrazones of keto acids have a tendency to form multiple spots. When investigating the keto acids of *Asplenium* we have therefore reduced the extracted dinitrophenyl hydrazones with tin in an alcoholic hydrochloric acid solution to the corresponding amino acids, which then have been determined by the paper chromatographic method using butanol-acetic acid and phenol + NH_3 as solvents. This method presupposes that the hydrazone extract is free from amino acids. Kulonen⁵ and Towers and Steward⁶ have recently reported that they have used a method of this kind for the determination of keto acids, the reduction being made with other agents.

Using the above-mentioned method we found α -ketoglutaric acid, pyruvic acid,

glyoxylic acid, hydroxypyruvic acid, α -ketopimelic acid, and γ -hydroxy- α -ketopimelic acid in *Asplenium septentrionale*, provided that the amino acids which appeared on the paper chromatogram were formed from the corresponding hydrazones. As only very faint spots of some amino acids, and no spots of serine, α -aminopimelic acid, and γ -hydroxy- α -aminopimelic acid were formed on the chromatogram made with the extract of non-reduced hydrazones the occurrence of the above-mentioned keto acids in *Asplenium* is to be considered sufficiently proved (Figs. 1 and 2). The amount of all the keto acids is so small that their isolation was impossible with the plant material we had at our disposal (ca. 500 g).

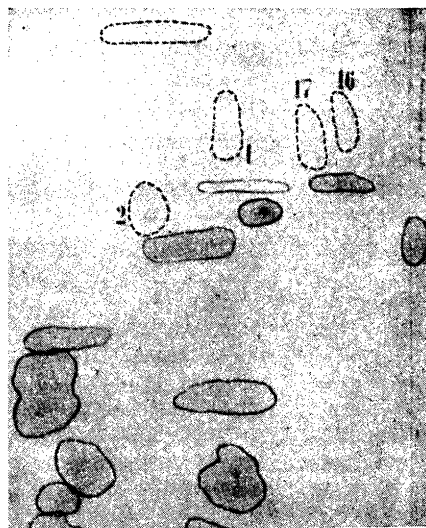


Fig. 1. Two-dimensional paper chromatogram (phenol + NH_3 and butanol-acetic acid) of the non-reduced 2,4-dinitrophenylhydrazones. Dot-lined spots = with ninhydrin produced very faint spots of 1 = gly, 2 = ala, 16 = asp, 17 = glu. Full lined spots = yellow hydrazone spots without ninhydrin treatment.

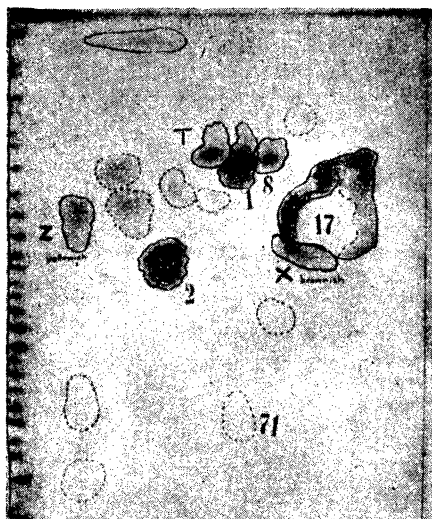


Fig. 2. Two-dimensional paper chromatogram of the reduced hydrazones. Full-lined spots = with ninhydrin produced distinct spots of 1 = gly, 2 = ala, 8 = ser, 17 = glu, X = γ -hydroxy- α -aminopimelic acid (brownish), Z = lactone of γ -hydroxy- α -aminopimelic acid (yellowish), T = unknown. Dot-lined spots = with ninhydrin produced faint spots. 71 = α -aminopimelic acid; unnumbered ninhydrin spots are unidentified.

Among the keto acids which we have found hydroxypyruvic acid, α -ketopimelic acid and γ -hydroxy- α -ketopimelic acid especially attract attention as they have not earlier been found in any plant as far as we know. In addition there are some ninhydrin positive unidentified spots on the chromatogram, among which spot T is particularly intense. The characterization of γ -hydroxy- α -ketopimelic acid has been possible only because the corresponding amino acid was earlier isolated from *Asplenium*¹.

In germinating pea seeds formation of α -ketoadipic acid could be shown. Even this acid has not earlier been found in any organism.

Our investigations suggest that α -amino-adipic acid, α -aminopimelic acid and γ -hydroxy- α -aminopimelic acid are formed *via* transamination from the corresponding keto acids. We are going to investigate these transamination reactions more closely. The formation of serine from hydroxy-

pyruvic acid, and the formation of glycine from glyoxylic acid seems also probable.

1. Virtanen, A. I. and Berg, A.-M. *Acta Chem. Scand.* **8** (1954) 1085.
2. Virtanen, A. I., Uksila, E., and Matikkala, E. J. *Acta Chem. Scand.* **8** (1954) 1091.
3. Virtanen, A. I., Miettinen, J. K. and Kunttu, H. *Acta Chem. Scand.* **7** (1953) 38.
4. Virtanen, A. I. and Laine, T. *Suomen Kemistilehti B* **10** (1937) 35; Virtanen, A. I. and Arhimo, A. A. *Nature* **144** (1939) 36; Virtanen, A. I., Arhimo, A. A. and Suomalainen, H. *Nature* **144** (1939) 597; Virtanen, A. I., Arhimo, A. A., Sundman, J. and Jännes, L. *J. prakt. Chem.* **162** (1943) 71.
5. Kulonen, E. *Scand. J. Clin. & Lab. Invest.* **5** (1953) 72.
6. Towers, G. H. N. and Steward, F. C. *J. Am. Chem. Soc.* **76** (1954) 1959; Towers, G. H. N., Thompson, J. F. and Steward, F. C. *J. Am. Chem. Soc.* **76** (1954) 2392.

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The Synthesis of Phenylcarbamoyl Derivatives by Lossen Rearrangement of Dibenzohydroxamic Acid

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Reports on the modification of proteins by reaction with organic isocyanates (notably phenyl isocyanate) have been published by numerous authors (for review, see Herriott¹). One common feature of these experiments has been the use of an excess of the reagent. The consequence of this has probably been the production of a very complex mixture of highly substituted protein derivatives and in most cases, when the reactions were performed in aqueous solution, hydrolysis of an uncontrolled amount of the reagent has occurred.

A method by which proteins and peptides in aqueous solution could be partially substituted by reaction with phenyl isocyanate under strictly controlled conditions would be highly desirable. It appeared to us that the well known Lossen rearrangement of dibenzohydroxamic acid might be useful in this respect.