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New α -Keto Acids in Green Plants.

II. β -Hydroxy- and γ -Hydroxy- α -Ketobutyric Acid in Cowberries

ARTTURI I. VIRTANEN and MAGNUS ALFTHAN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

Using the method mentioned in our earlier paper¹ and in detail described in a later paper² we have identified a couple of new α -keto acids in some berries and green plants.

In berries of cowberry (*Vaccinium vitis idaea*) we identified the following α -keto acids (Fig. 1) (in brackets the amino acids formed by hydrogenolysis of the 2,4-dinitrophenylhydrazones of the corresponding keto acids): oxalacetic acid (aspartic acid), α -ketoglutaric acid (glutamic acid), hydroxypyruvic acid (serine), glyoxylic acid (glycine), γ -hydroxy- α -ketobutyric acid (homoserine), β -hydroxy- α -ketobutyric acid (threonine), and pyruvic acid (alanine). β - and γ -hydroxy- α -ketobutyric acid are new α -keto acids which have not earlier been found in any organism. The relatively intense spots T and Y represent unknown amino acids, and the corresponding keto acids are therefore also unknown.

If we assume that about 60% of the keto acids² found in fresh cowberries (10.2% dry substance, 0.93% tot. N of dry subst., 10.5% sol. N of tot. N) are reduced to amino acids when our method

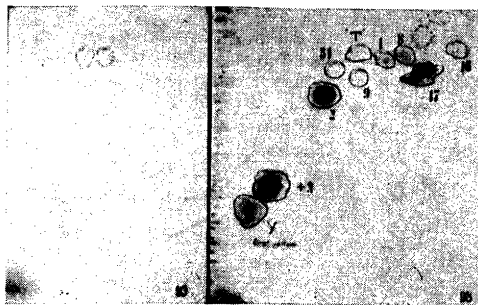


Fig. 1. Berries of *Vaccinium vitis idaea*. Left: Two-dimensional paper chromatogram (phenol-NH₃ and butanol-acetic acid) of the non-reduced 2,4-dinitrophenylhydrazones. Practically no spots of amino acids. Right: Two-dimensional paper chromatogram of the reduced hydrazones. 1 = gly, 2 = ala, 8 = ser, 9 = threo, 16 = asp, 17 = glu, 51 = homoser, T and Y = unknown amino acids. + 3 = valine added.

is used, the amount of different keto acids in 100 g of fresh cowberries are roughly the following: oxalacetic acid 25 μ g, α -ketoglutaric acid 220 μ g, pyruvic acid 130 μ g, hydroxypyruvic acid 40 μ g, glyoxylic acid 30 μ g, γ -hydroxy- α -ketobutyric acid 20 μ g, and β -hydroxy- α -ketobutyric acid 10 μ g. As found by Alfthan and Virtanen² the 60% yield which has been the basis for these calculations does not hold good for all amino acids. As, however, only yields



Fig. 2. Berries of *Oxycooccus quadripetalus*. Left: Two-dimensional paper chromatogram of the non-reduced 2,4-dinitrophenylhydrazones. Practically no spots of amino acids. Right: Two-dimensional paper chromatogram of the reduced hydrazones. Note the very strong spot of 17.

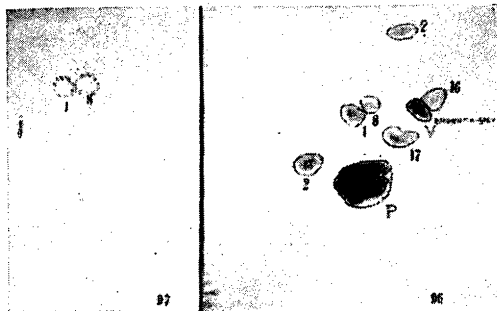


Fig. 3. *Phyllitis scolopendrium*. Left: Two-dimensional paper chromatogram of the non-reduced 2,4-dinitrophenylhydrazones. Practically no spots of amino acids, except extremely weak spots of 1 and 8. Right: Two-dimensional paper chromatogram of the reduced hydrazones. In addition to 1, 2, 8, 16, 17 the unknown amino acids V and P.

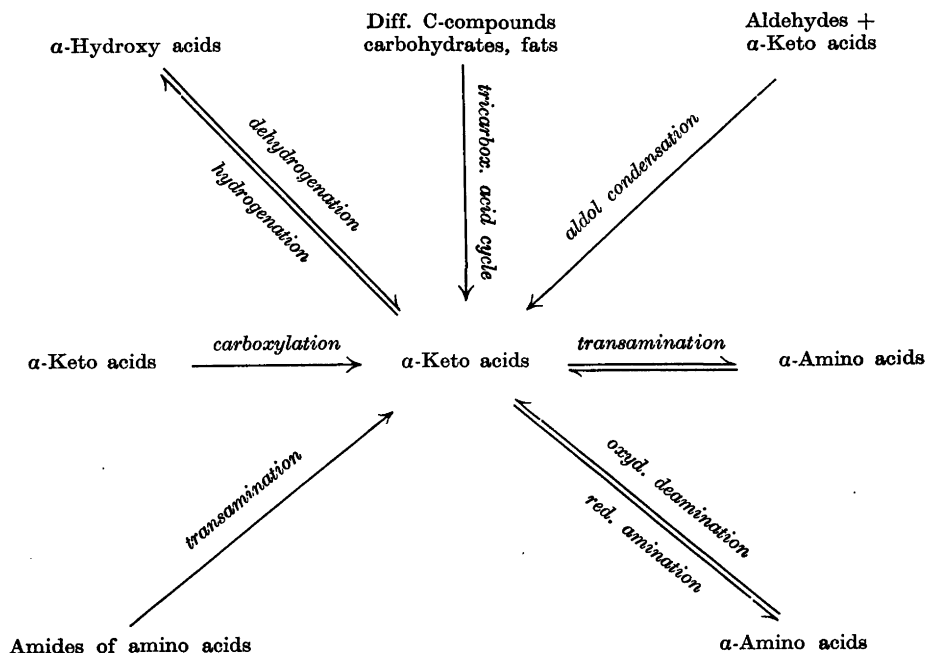
of a few amino acids formed from keto acids are known we have used the above mean yield.

In berries of mossberry (*Oxycoccus quadripetalus*) the keto acids are qualitatively

nearly the same as in cowberry (Fig. 2). The keto acid corresponding to threonine was not, however, to be found in cowberry, at least not in the concentration used. The keto acid corresponding to the unknown amino acid Y was also lacking. The great amount of α -ketoglutaric acid in mossberry is especially remarkable.

Using the same basis for calculation as before the amount of different keto acids in mossberry (100 g of fresh berries) are roughly the following: oxalacetic acid 50 μ g, α -ketoglutaric acid 2 700 μ g, pyruvic acid 130 μ g, hydroxypyruvic acid 25 μ g, and glyoxylic acid 35 μ g.

In a fern (*Phyllitis scolopendrium*) Virtanen and Berg³ have found very large amounts of an unknown acidic OH-containing amino acid P, and a smaller amount of another amino acid V. (cf. Fig. 3). These authors are going to give a more detailed account of the isolation, structure, and properties of these new amino acids. Our keto acid determinations show (Fig. 3) that the keto acid corresponding to the amino acid P occurs very abundantly in this plant as compared with other keto acids, as, according to Virtanen and Berg, the amount of this amino acid is also by far the greatest when compared with other free



amino acids. The amount of the keto acid corresponding to the amino acid V is much smaller as is also that of the amino acid V in comparison with other free amino acids.

It is often to be noted that a great amount of an amino acid corresponds to a great amount of keto acid, at least when uncommon free amino acids are in question. This gives support to the conception that these amino acids may be formed from the corresponding keto acids *via* transamination.

The occurrence of a number of different α -keto acids corresponding to different amino acids in plants is due to the fact that these keto acids are either precursors to, or deamination products of, the corresponding amino acids. If deamination occurs *via* transamination the keto acids are both deamination products and precursors because the reaction is reversible, the equilibrium depending on the concentration of keto and amino acids participating in the transamination. Also the keto acid formed *via* oxidative deamination can be transformed into the corresponding amino acid *via* transamination. From amino acids which are formed from other

amino acids *via* specific reactions (as *e. g.* homoserine from aspartic acid, threonine from homoserine *etc.*) the corresponding keto acid can be formed *via* deamination, and from this again *via* transamination the corresponding amino acid. The following scheme presents different possible pathways for the formation of α -keto acids in organisms and also for the formation of amino acids and amides, respectively, from α -keto acids.

The appearance of numerous widely different α -keto acids in plants gives further proof for the importance of these acids in plants.

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