

## Bound Homoserine in Fruits of Cowberry and Cranberry

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The numerous new amino acids, found in recent years, generally occur as free amino acids. Only  $\gamma$ -hydroxyglutamic acid is found in bound form too in *Phlox*<sup>1</sup> and  $\alpha$ -amino adipic acid in proteins in maize<sup>2</sup>. Homoserine, which is a comparatively common free amino acid of plants and which is formed in germinating pea seeds in a surprisingly large quantity<sup>3</sup>, has not been simultaneously found in free and bound form, whereas we have found it in bound form alone in berries and leaves of cowberry<sup>1</sup>.

Fruits of cowberry were extracted with 70 % ethanol until no ninhydrin positive spots could be found on the paper chromatogram. The free amino acids in the ethanol extract can be seen in Fig. 1. No homoserine and hydroxyproline could be found on the chromatogram.

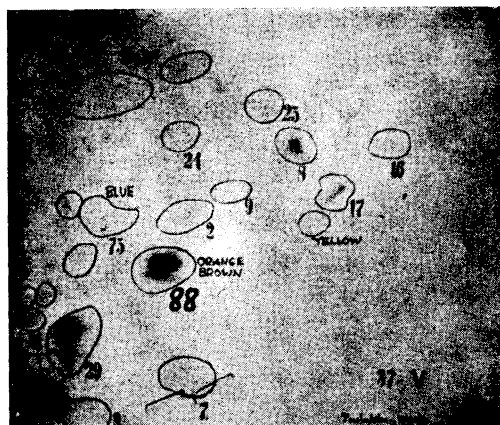


Fig. 1. Free amino acids of fruits of cowberry on a two-dimensional chromatogram (butanol-acetic acid-water, and phenol-water-NH<sub>3</sub>; same solvents in Fig. 2). 2 = ala, 3 = val, 7 = tyr, 8 = ser, 9 = threo, 11 = pro, 16 = asp, 17 = glu, 24 = glu-NH<sub>2</sub>, 25 = asp-NH<sub>2</sub>, 29 =  $\gamma$ -aminobut. acid, 60 = pipercolic acid, 75 = 5-hydroxypipercolic acid, 88 = new cyclic  $\alpha$ -aminocarboxylic acid (1-cyclopropane-1-aminocarboxylic acid).

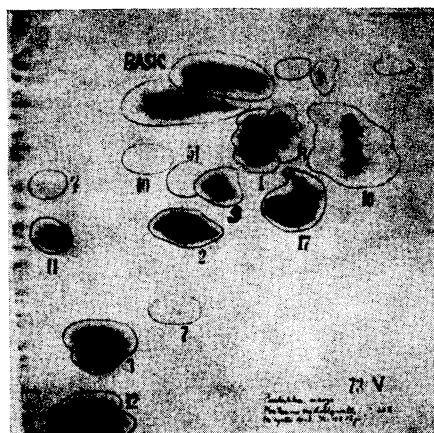


Fig. 2. Paper chromatogram of the hydrolyzed residue of 70 % ethanol extract of fruits of cowberry: 1 = gly, 10 = hydroxyproline, 51 = homoserine.

After ethanol extraction the residue of the berries was dried. 1 g of the residue was hydrolyzed with 15 ml of 6 N HCl for 24 h at 108 C°. Amino acids were separated in an Amberlite IR-120 column. Clear spots of homoserine and hydroxyproline were

found on the paper chromatogram (Fig. 2). On the other hand  $\alpha$ -aminobutyric acid (29), pipercolic acid (60), 5-hydroxypipercolic acid (75), and 1-cyclopropane-1-aminocarboxylic acid (88) which occur as free amino acids in the berries are not found on the paper chromatogram. Also in berries of cranberry (*Oxycoccus quadripetalus*) homoserine was found in bound form.

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## Disubstitution of Ethyl Acetoacetate in one Step by Means of Sodium Hydride

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Hitherto it has not been considered possible to prepare the disubstituted derivatives of acetoacetic ester in one step. For instance, Finar<sup>1</sup> points out that "the introduction of two alkyl groups must be carried out in two steps; it *cannot* be done in one step". However, Wallingford and Homeyer<sup>2,3</sup> have reported that dimetallation and disubstitution of  $\beta$ -ketoesters is possible with alcohol-free sodium alkoxide in alkyl carbonate solution, and Weizmann<sup>4</sup> was able to dialkylate acetoacetic ester by means of a complex formed between potassium hydroxide and an acetal or dialkyleter of ethylene glycol.

The present writer has found that ethyl  $\beta$ -acetotricarballylate can be prepared in good yields from ethyl acetoacetate and ethyl bromoacetate, using sodium hydride as metallating agent in benzene solution.

It is the author's intention to further investigate the extent to which this method is applicable.

*Experimental:* The apparatus used was a three-necked flask, fitted with a dropping funnel, a sealed Hersberg stirrer and a condenser, the top of which had connections to a source of nitrogen and a vacuum line. The apparatus was evacuated, flame-dried and filled with nitrogen. With nitrogen flowing, the dropping funnel was closed and removed, and sodium hydride (28.8 g, 1.2 mole) rapidly placed in the flask and covered with benzene (700 ml). The funnel was then replaced, the flow of nitrogen stopped and the funnel filled with a solution of freshly distilled ethyl acetoacetate (65 g, 0.5 mole) in dry benzene (150 ml) and then stoppered with a calcium chloride tube. With stirring, this solution was added during 30 min and the reaction mixture then stirred until the evolution of hydrogen had stopped. The apparatus was now swept with nitrogen, before a solution of freshly distilled ethyl bromoacetate (200 g, 1.2 mole) in dry benzene (75 ml) was placed in the dropping funnel. This solution was added with vigorous stirring during two hours, the flask being gently heated to maintain a slight reflux (the reaction is exothermic). When the addition was finished, the reaction mixture was stirred and refluxed for another 45 min.

After cooling, the reaction mixture was washed three times with water, the water washings extracted once with ether and the combined benzene and ether solutions dried over anhydrous magnesium sulphate. The solvents were removed and the residual oil fractionated, yielding 116.5 g (77 %) of  $\beta$ -acetotricarballylate, b. p. 142°—47°/1.0—1.2 mm. Phenylhydrazone m. p. 99°—100.5°. Emery<sup>5</sup> reports 190°/16 mm and 100°—101°, respectively.

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