

Free Sarcosine in Reindeer-Moss (*Cladonia silvatica*)

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During investigation of free amino acids in plants we have found sarcosine in reindeer-moss (*Cladonia silvatica*). As far as we know this N-methyl-glycine has not earlier been observed in a free state in plants. Haworth *et al.*¹ have, however, found that sarcosine is formed by acid hydrolysis of protein separated from the groundnut. If the protein preparation investigated did not contain other components this would be the first time sarcosine has been found as a constituent of a protein molecule. Plattner and Nager² have found that the antibiotic enniatines contain N-methyl-L-isoleucine,³ N-methyl-L-valine, and N-methyl-L-leucine. Dalglish *et al.*⁴ have found sarcosine and N-methyl-L-valine in their "Antibiotic X-45". These N-methyl-amino acids are also components

of the actinomycin C of Brockmann *et al.*⁴ Participation of N-methyl-amino acids in the formation of some antibiotics is thus proved.

The isolation of the free amino acids was accomplished in accordance with earlier methods used in this laboratory. An extract of ethanol, which was made by crushing 500 g of fresh moss in ethanol (alcohol concentration after extraction being about 70%), and keeping the mixture for 6 days in a refrigerator, had a volume of 2 000 ml. This was passed through a column containing 40 g Amberlite 120. The amino acids remain in the resin, and were displaced by 300 ml of 1 N ammonium hydroxide. Using a fraction-collector, and collecting fractions of 10 g, amino acids were mainly found in fractions 12–16. The fractions were of a reddish brown colour.

In fractions 12–16 the following amino acids were found through two dimensional paperchromatographic analysis (butanol-acetic acid and phenol in NH₃-atmosphere): aspartic acid, glutamic acid, serine, glycine, asparagine, glutamine, threonine, alanine, proline, lysine, arginine (?), sarcosine, α -amino-n-butyric acid, γ -aminobutyric acid, tyrosine, valine, isoleucine, phenylalanine. In addition ethanol-amine was found.

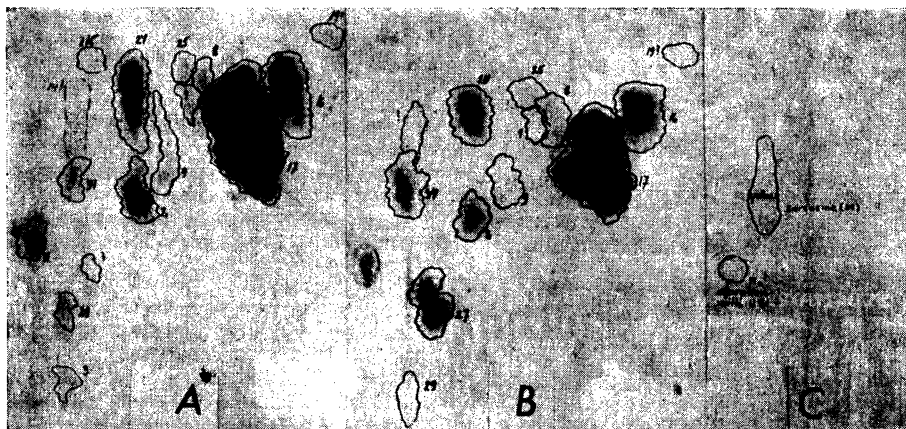


Fig. 1. Free amino acids in *Cladonia silvatica*. Two-dimensional chromatograms. A = extract, spots with ninhydrin, B = extract + addition of sarcosine and α -aminobutyric acid, spots with ninhydrin, C = extract with p-nitrobenzoylchloride in toluene solution. 1 = gly, 2 = ala, 3 = val, 8 = ser, 9 = thr, 11 = pro, 14 = arg, 15 = lys, 16 = asp, 17 = glu, 24 = glu NH₂, 25 = asp NH₂, 27 = α -amino-n-butyric acid, 29 = γ -aminobutyric acid, 34 = sarcosine.

The identification of sarcosine is founded on the following observations:

1. The colour given by ninhydrin is typical for sarcosine.
2. The spot of added sarcosine coincided with the spot given by the plant extract.
3. With *p*-nitrobenzoylchloride the spot gave a yellow colour².
4. When α -amino acids were deaminated with nitrous fumes (from NaNO₂ and dilute HCl) only spots of sarcosine (characteristic colour) and proline developed when spraying the paperchromatogram with ninhydrin³.

When the plant residues after ethanol extraction were hydrolyzed with acid, sarcosine was not to be found by the paperchromatographic method. Accordingly, the proteins did not contain sarcosine.

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Carbethoxylation of 2-Thenyl Cyanide

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In an earlier paper the present author has described the carbethoxylation of 2-thenyl cyanide by a general process¹. This paper seems to have been overlooked by Leonard and Simet, who about a year ago described a modified procedure². During the last three years a very similar method has been used at this laboratory for the preparation of ethyl 2-thienylecyanoacetate. The differences appear to be of some interest from the practical point of view, however, and are therefore reported here.

Brändström has described a method for the carbethoxylation of ketones, esters and nitriles, using sodium ethylate (prepared from sodium and ethyl carbonate) as condensing agent, and ethyl carbonate as solvent³. A slight modification of this method (alcohol being removed *in vacuo*) gives a good yield of ethyl 2-thienylecyanoacetate.

The above-mentioned paper¹ contains a reference to a paper by Wideqvist⁴, which perhaps gives the solution of Leonard and Simet's problems concerning the hydrolysis of the alkylated 2-thienylecyanoacetates.

Experimental: 200 ml of dry ethyl carbonate was placed in a 500 ml, three-necked, round-bottomed flask, fitted with a dropping funnel, a glycerol-sealed stirrer and a Widmer column, connected to a distillation condenser. The flask was heated in an oil bath until it boiled gently. The oil bath was removed, and 3.2 g (0.14 moles) of sodium was added to the hot, stirred solution at such a rate that refluxing occurred. When all the sodium was dissolved, the mixture was allowed to cool to about 80°, and the apparatus was evacuated to a pressure of about 300 mm Hg. 16.0 g (0.13 moles) of 2-thenyl cyanide was then added in a rapid stream to the mixture in the flask. The alcohol was removed, and when the temperature at the top of the column attained the expected boiling point of ethyl carbonate, the oil bath was removed and the flask allowed to cool. The contents were poured over a mixture of 20 ml of glacial acetic acid and ice. The organic layer was separated and the water layer extracted with ether. The combined organic layers were washed with water, dried over anhydrous sodium sulphate, and the ether removed. The residue was finally fractionated *in vacuo*. The yield of ethyl 2-thienylecyanoacetate, boiling at 115°–120°/1–2 mm, was 17.7 g (70 %).

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