ACTA CHEMICA SCANDINAVICA 11 (1957) 741—756

Short Communications

A New Cyclic α-Aminocarboxylic Acid in Berries of Cowberry

MAIJA-LEENA VÄHÄTALO and
ARTTURI I. VIRTANEN

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

In 1955, in a lecture on new amino acids isolated in this laboratory, one of us [1] presented a two-dimensional paper chromatogram on which also the presence of a new amino acid (No. 88 in this laboratory) was indicated: "α-Aminosäure isoliert aus Preisselbeeren; Struktur noch nicht sichergestellt". This amino acid is dealt with in the present paper.

Isolation of the amino acid 88. About 12 kg (fresh weight) of cowberries were crushed and amino acids were extracted from them with 70 % ethanol (the water content of the berries taken into account). The extract was concentrated and passed through an Amberlite IR-120 column. Amino acids were eluted from the resin with 1 N ammonium hydroxide. The eluate was evaporated to dryness *in vacuo* and the residue (very dark) dissolved in 20 ml distilled water. It was then passed through an Amberlite IR-4B column. Neutral amino acids were removed from the column by elution with water, while acidic amino acids remained in the resin. On a two-dimensional paper chromatogram (solvents: *n*-butanol--acetic acid-water (63:10:27) and phenol water-ammonia) an unknown spot appeared below alanine among neutral amino acids. On drying at 70° C the spot was yellow but soon turned orange-brown and then violet. The spot was unaffected by hydrolysis with 6 N HCl. It disappeared on deamination with nitrous oxides and was therefore apparently an α-amino acid.

The eluate was evaporated to dryness and the residue dissolved in a small volume of 1.5 N HCl. The new amino acid emerged in fractions 375—455 (of 10 ml each) together with valine and proline. The fractions were evaporated to dryness and suspended in butanol, to which cellulose powder was added. The dry powder obtained was sprinkled on the top of a cellulose powder column. The unknown amino acid was eluted by *n*-butanol from the cellulose powder column, by which treatment valine was separated. Finally the amino acid was separated from proline on a one-dimensional paper chromatogram, using phenol-NH_3 as solvent. The zone containing the amino acid 88 was cut out from the papers and the paper strips were extracted with distilled water. The residue, evaporated to dryness, was passed through an Amberlite IR-120 column. It was crystallized from hot ethanol. Almost white needle-shaped crystals were formed. The crystals were very soluble in water but not in absolute ethanol, acetone or ether.

Characteristics of the amino acid 88: R_F-values (Whatman No. 4):

	Phenol-water-NH_3	*n*-BuOH-AcOH-water
88	0.67	0.33
Alanine	0.62	0.25

Total hydrolysis (6 N HCl, 24 h, 108 °C): Spot stable, traces of homoserine (51) are formed. *Alkaline hydrolysis* (saturated $Ba(OH)_2$, 1 h, 100 °C; 10 % NaOH, 2 h, 108 °C, 5 % Na_2CO_3, 2 h, 108 °C): Stable. *Deamination* with nitrous oxide: Spot disappears (as controls: proline, γ-aminobutyric acid and 5-oxypiperidine-2-carboxylic acid which do not disappear); the acid is an α-amino acid. *Oxidation* with $KMnO_4$ in acid solution (4 % $KMnO_4$ solution, 20 %

Fig. 1. Two-dimensional paper chromatogram of amino acid 88 after reduction with HI and red P (butanol-acetic acid-water, and phenol-water-NH_3; same solvents in Fig. 2). Alanine (2) and valine (3) added. 51 = homoser, 81 = lactone of homoser, 27 = α-aminobut. acid.

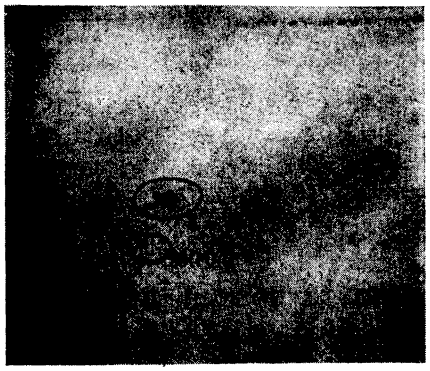

Fig. 2. Paper chromatogram of amino acid 88 after catalytic reduction. Alanine (2) and valine (3) added. 27 = α-aminobut. acid.

H_2SO_4) at +80 °C and with H_2O_2 at room temperature: No new amino acids were found on the paper chromatogram; part of the amino acids remained unoxidized. *Reduction* with HI and red P (140 °C, 1.5—4 h): Homoserine and its lactone as well as a small spot in the position for α-amino-*n*-butyric acid are formed (Fig. 1). Depending on circumstances either homoserine or the lactone of homoserine or both were formed. The reduction of pure homoserine gave a similar result as the reduction of 88. *Catalytic reduction* with PtO_2 and H_2 (Adams platinum oxide catalyst B 27, 6 h): α-NH_2-*n*-Butyric acid (27) is formed as well as faint spots in the position for alanine and in the vicinity of valine (Fig. 2). *UV spectrum:* Non-aromatic; resembling the uncharacteristic spectra of aliphatic amino acids. *Double bond:* Did not reduce alkaline $KMnO_4$ solution; no double bond. *Microtests:* The establishing of lactones as ferrihydroxamate; negative. The OH group in α-position to the amino group (Nessler); negative. The aldehyde or keto group (with 2,4-dinitrophenylhydrazine); negative. Isatin spraying (proline and its homologue); negative. Vanilline spraying; negative.

The above information on the new amino acid was obtained in the autumn of 1954 and in the winter of 1955. On the basis of the results we knew even then that the amino acid had a cyclic structure, and that homoserine or α-aminobutyric acid were formed from it on treatment with different reducing agents. The ring was very stable on hydrolysis, differing thus from the cyclic imino acid isolated from *Convallaria majalis* and *Polygonatum officinale*, respectively [2-4] (2-azetidinecarboxylic acid [3,4]). On paper chromatograms both substances also behaved differently. C, H, and N determinations of the new amino acid suggested a formula $(C_4H_{7-8}O_2N)_n$ but the molecular weight determination was lacking. At this stage the investigation was broken off because the younger author had to give up her research work for the time being and the amount (about 20 mg) of the pure amino acid isolated was used up.

In the autumn of 1956 the investigation was resumed. About 30 mg of the amino acid were isolated and the following additional information was obtained: *Microanalysis.* Found: C 47.62; H 6.89; N 13.91; O 32.59 (determ.). Calc. for $C_4H_7O_2N$: C 47.52; H 6.99; N 13.86; O 31.68. *Molecular weight* (Child's method): Values between 90 and 100 were found in different determinations; Calc. 101; n = 1. *Formation of CO_2 and NH_3* by ninhydrin (Linko's method): CO_2 is not formed, NH_3 to only about 30 % of the total N. Bromine is not taken up; no double bond. *Melting point:* Decomposes at about 234—236 °C. *Microtests:* Waser-Edlbacher (—N—CH_3); negative. Sodium nitroprusside + acet-

aldehyde . (secondary aliphatic amines); negative. α-Naphthoquinonesulphonate (secondary amino group):

	Solution A	Solution B
proline	blue	red
alanine	blue	blue
88	red	red

When at the end of February 1957 the molecular weight of the amino acid was established there were not many possibilities left for structure speculation. Then a paper was published by Burroughs [5] in which the isolation of 1-amino-*cyclo*propane-1-carboxylic acid from perry pears and cider apple was reported. The composition and characteristics of this amino acid are in complete agreement with the amino acid 88 isolated by us from cowberries. Any other structure than that presented by Burroughs cannot be given for the amino acid. Burroughs has also confirmed the structure of his amino acid by synthesis.

In August and September 1955 Mrs. Onerva Äyräpää performed isolation experiments of the amino acid 88 with ripe and unripe (on the one hand partly red and on the other hand quite green) cowberries. The amino acid was not found in unripe berries. The situation is thus the same in cowberries and in perry pears [5].

The content of the amino acid 88 in ripe cowberries is, according to paper chromatography determination, about 400 μg per 100 g fresh wt. and 3.2 mg per 100 g dry wt. or 0.4 % of ethanol soluble N.

Added in proof. Comparison of our preparation with a sample of Burrough's kindly sent by him has conclusively confirmed their identity.

1. Virtanen, A. I. *Angew. Chem.* **67** (1955) 381, Fig. 5.
2. Virtanen, A. I. and Linko, P. *Acta Chem. Scand.* **9** (1955) 551.
3. Fowden, L. *Nature* **176** (1955) 347.
4. Virtanen, A. I. *Nature* **176** (1955) 984.
5. Burroughs, L. F. *Nature* **179** (1957) 360.

Received March 23, 1957.

Paper Chromatography of Amino Acids, Sugars and Acids

GUNNAR HÖGSTRÖM

Dept. of Physiological Chemistry, Wenner Grens Institute, Stockholm, Sweden

The solvents commonly used for the paper chromatography of amino acids, namely phenol, collidine and butanol-acetic acid, popular for their good separatory powers, have several disadvantages: phenol and collidine are toxic, and the butanol-acetic acid mixture is subject to esterification with concomitant change of R_F-values. Their smell is also rather offensive. Many attempts have been made to replace them with less obnoxious solvents. In this article will be described an attempt to use methyl ethyl ketone in mixtures with acetone, water and formic acid for the separation of amino acids. These solvents have also been used successfully for sugars and acids belonging to the citric acid cycle.

Experimental: Methyl ethyl ketone of puriss and acetone of pure grade is used. No purification is necessary. The chromatography chambers are made of glass, $20 \times 30 \times 60$ cm, with glass-lids and glass-troughs supported on a glass-frame. Since the R_F-values of amino acids and sugars are very low in the solvents described here, a relatively fast paper, such as Whatman 4, has been used for these compounds, and its lower edge serrated so as to allow the solvent to drip off easily. Whatman 1 is used for the acids. The amino acids are developed by dipping the chromatogram in a 0.2 % solution of ninhydrin in acetone with 0.1 % acetic acid and a little collidine to bring out the right colours of the spots. The sugars are developed with silver nitrate or aniline hydrogen phthalate. The acids are developed by dipping in an indicator solution prepared from a 1 % solution of bromophenol blue in 95 % alcohol by neutralizing to the first tinge of red and then adding about five times its volume of petroleum ether.

In water-saturated methyl ethyl ketone alone the R_F-values of amino acids and sugars are too low to be practical. The addition of acetone makes it possible to introduce more water and increase the