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 Burroughs⁵ in which the isolation of 1-amino-cyclopropane-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 pears⁵.

The content of the amino acid 88 in ripe cowberries is, according to paper chromatography determination, about 400 μ g 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

Fig. 1. Paper chromatogram of amino acids.

R_F -values. The following procedure has turned out to be useful for the amino acids:

On a 13 cm wide paper strip the unknown sample is applied on the starting-line 3 cm from one end and a mixture of the common amino acids 2 cm from the other end (Fig. 1). The chromatogram is run for about 30 h with a mixture of methyl ethyl ketone, acetone and water in the proportions 3:1:0.6 (solvent I) and dried at room temperature. A 4 cm wide strip containing the control mixture is separated from the main strip and developed with ninhydrin. The fastest amino acid, phenylalanine, will have travelled

about 40 cm. The positions of proline and valine on the sample strip can be found with the aid of the control strip and the sample strip is cut between these two amino acids. The distal part is developed with ninhydrin. A new strip of paper is sewed on the undeveloped part at right angles to the direction of the first solvent and the remaining amino acids separated on this strip with solvent II — methyl ethyl ketone, acetone, water and formic acid in the proportions 3:1:1:0.1 — during 30 h. Here glutamic acid is the fastest amino acid. If the R_F -value of phenylalanine in solvent I and glutamic acid in solvent II is arbitrarily designed as 1.00, the other amino acids will obtain the R_F -values collected in Table 1.

For sugars solvent I is used. Of the common sugars ribose is the fastest one and travels about 40 cm in 16 h. If its R_F -value is designed as 1.00, the other sugars will get the R_F -values collected in Table 2.

A mixture of methyl ethyl ketone, acetone, water and formic acid in the proportions 80:6:12:2 is convenient for the separation of some common acids, the R_F -values of which are found in Table 3. On Whatman 1 the front travels about 40 cm in 6 h.

Instead of solvent I a mixture with the proportions 15:3:2 has sometimes been used with a little longer running time and somewhat improved separation. In this solvent tryptophan runs ahead of phenylalanine — in solvent I they coincide.

Salts in appreciable amounts have a pronounced effect on the separatory powers of these solvents, at least with respect to the amino acids. The only natural material, to which they have been applied so far, is acid-hydrolyzed *Neurospora*, and in this case this drawback has been obviated

Table 1. R_F -values of amino acids.

Solvent	I	II		I	II
Tryptophan	1.00		Hydroxyproline	0.19	0.73
Phenylalanine	1.00		Serine	0.16	0.64
Leucine	0.94		Glycine	0.14	0.73
Isoleucine	0.80		Histidine	0.09	0.28
Methionine	0.70		Citrulline	0.11	0.55
Tyrosine	0.67		Aspartic acid	0.04	0.74
Valine	0.48		Glutamic acid	0.04	1.00
Proline	0.28		Arginine	0.04	0.44
Alanine	0.21	1.00	Lysine	0.02	0.30
Threonine	0.21	0.82	Ornithine	0.02	0.25

Table 2. R_F -values of sugars.

Rhamnose	1.13
Ribose	1.00
Xylose	0.72
Arabinose	0.57
Fructose	0.52
Sedoheptulose	0.52
Mannose	0.50
Glucose	0.39
Mannoheptulose	0.39
Galactose	0.32
Lactose	0.09

Table 3. R_F -values of acids.

Fumaric acid	0.88
Aconitic acid	0.85
Succinic acid	0.83
Pyruvic acid	0.72
Lactic acid	0.72
α -Ketoglutaric acid	0.65
Malic acid	0.54
Isocitric acid	0.52
Citric acid	0.49
Glyceric acid	0.37
Tartaric acid	0.29
Oxalic acid	0.12

by using a simple desalting apparatus, illustrated in Fig. 2. The sample is applied along a line 5 cm from one end of a paper strip, 21 $\times$ 5 cm, pointed at the other end. The strip is placed between two glass sheets, 16 $\times$ 6 cm, in such a way that the pointed end is 1 cm outside the sheets. The other end is immersed in a mixture of acetone, water and ammonia in the proportions 7:3:0.4. After about 8 h all the amino acids have travelled to the pointed end of the strip, leaving most of the salts behind. The end is cut off and eluted with water. The eluate is concentrated and applied for chromatography. The application can, more conveniently, be made automatically by allowing the pointed end of the paper strip to touch the starting-line of

Fig. 2. Desalting apparatus.

the chromatography strip. A blast of air, directed from underneath this strip, accelerates the evaporation and prevents the spot from becoming too big.

About 20 cm has been found to be enough for the desalting strip in the case of *Neurospora*. Other materials may require greater length and time of desalting.

My thanks are due to Professor G. Ehrensvärd for much encouragement in this work.

Received April 10, 1957.

The Reflection Spectra of Anhydrous Nickel(II) Chlorides and Bromides and Their Double Salts with Monovalent Cations

R. W. ASMUSSEN and OLE BOSTRUP

Chemical Laboratory B, Technical University of Denmark, Copenhagen, Denmark

It is generally assumed that the nickel ions in anhydrous NiCl_2 , NiBr_2 and their double salts MeNiCl_3 and MeNiBr_3 have the coordination number six. The magnetic properties of the double salts have previously been measured by Asmussen and Soling¹. The reflection spectra of ten solid compounds are given in Fig. 1 and in Table 1. The excited levels corresponding to the absorption maxima agree with the ligand field theory for d^8 -systems in fields of the cubic-octahedral symmetry O_h . Thus a ligand field parameter Δ (also denoted by $(E_1 - E_2)$ or $10Dq$) can be found for each compound. Further the Slater-Condon-Shortley integrals F^k seem to be decreased² in the complexes relative to the Ni^{2+} .

Fig. 1 shows that the ten absorption spectra are remarkably similar. However, the values of Δ given in Table 1 vary within a range of 6 800–7 600 K. Δ decreases with increasing radius of the anion (Cl^- , Br^-) and with increasing radius of the cation (Rb^+ , Cs^+ , $\text{C}_6\text{H}_5\text{NH}^+$). It has previously been discussed³ whether this behaviour can be explained by an electrostatic model. However, it is very probable that partly covalent bonding, *i. e.* intermixing of the partly filled 3d-shell with the orbitals of the ligands, occurs. The latter