passed through both suspensions in a Kluyver flask. In the control the nutrient solution containing the hydroxylamine fraction but no bacteria was aerated. The results can be seen in Fig. 2.

These results indicate that the hydroxylamine fraction is stable without *Azotobacter* during the experimental period, *i. e.* for 6 h, and that in experiments with *Azotobacter* bound hydroxylamine disappears much faster at pH 7.3 than at pH 6.5. The results suggest accordingly that the absence of bound hydroxylamine in *Azotobacter* cultures at pH 6.5 is due to a reduced formation of hydroxylamine compounds.

Since hydroxylamine compounds are formed in our *Azotobacter* cultures from NH_4—N as rapidly as from N_2, and identical compounds seem to be in question (*cf.* paper II), there is no evidence any longer of an oxidative step in aerobic N_2 fixation. On the basis of their results with *Clostridium* Virtanen and Hakala [5] years ago suggested that N_2 fixation by anaerobic organisms is a pure reduction, di-imide being the first intermediate, and the same mechanism can now be suggested also for aerobic N_2 fixation.

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On Hydroxylamine Compounds in *Azotobacter* Cultures. II. On the Chemical Nature of the Bound Hydroxylamine Fraction in *Azotobacter* Cultures

NILS-ERIK SARIS and ARTTURI I. VIRTANEN

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

In the previous communication [1] it was reported that bound hydroxylamine was accumulated in the nutrient solution of *Azotobacter*. Attempts to isolate and characterize these hydroxylamine compounds are reported in this paper.

Behaviour on ion exchange columns. Bound hydroxylamine from the nutrient solution passed through a slightly basic resin (Amberlite IR-4B), but was absorbed by a strongly basic one (IR-400). It was eluted quantitatively with diluted acetic or formic acid from the latter. On the other hand it was also absorbed almost quantitatively by acid resins from more concentrated solutions. Only some per cent of the total bound hydroxylamine passed through the Amberlite IR-120 column. From this column the amino acids and part of the hydroxylamine compounds could be eluted by 1 N ammonia, the latter compounds quantitatively only with 10 % ammonia. Paper chromatographic characteristics showed that the bound hydroxylamine eluted together with amino acids by dilute ammonia was similar to that eluted by more concentrated ammonia.

Separation of the bound hydroxylamine absorbed by the Amberlite IR-120 column. The fraction obtained from the glycerol cultures (N_2 as source of nitrogen) of *Azotobacter* was investigated by paper chromatography. The fraction was obtained by evaporating the culture solution to a small volume *in vacuo*, precipitating mucous substances with ethanol, evaporating ethanol *in vacuo* from the clear solution, passing the solution through the Amberlite IR-120 column, eluting the column

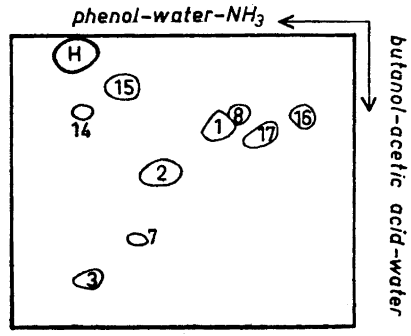

Fig. 1. Two-dimensional run of amino acids and bound hydroxylamine (formed in the culture solution of *A. chroococcum* K 1 grown in glycerol-flask-cultures for 3 days) after absorption in Amberlite IR-120 resin and elution with 1 N ammonia. H = bound hydroxylamine (a weak greyish spot with ninhydrin), 1 = gly, 2 = ala, 3 = val, 7 = tyr, 8 = ser, 14 = arg, 15 = lys, 16 = asp, 17 = glu.

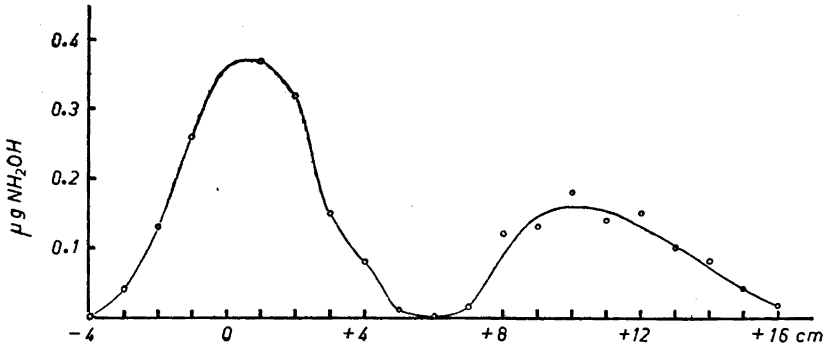

Fig. 2. Paper electrophoresis of bound hydroxylamine, pH 6.5, pyridine-acetic acid buffer, 1 000 V, 10 mA, 4 h.

with 10 % ammonia, upon which both amino acids and bound hydroxylamine went into solution quantitatively, and evaporating ammonia *in vacuo*. Phenol-water-ammonia and butanol-acetic acid-water were used as solvents. In a one-dimensional run the paper sheets were cut crosswise into narrow strips and hydroxylamine was determined from the water extracts after hydrolysis. The following R_F-values for bound hydroxylamine were found: 0.8 with phenol-water-ammonia, ~0.02 with butanol-acetic acid-water as solvents. (In parallel runs made for comparison the R_F-values for β-aspartylhydroxamic and γ-glutamylhydroxamic acids with butanol-acetic acid as solvents were 0.09 and 0.15.) Fig. 1 shows the position of bound hydroxylamine on a two-dimensional paper chromatogram. Independently of the nitrogen source (N_2-, NH_4-, NO_3—N) the same bound hydroxylamine spot was found on paper chromatograms. The spot was slightly ninhydrin positive and could thus be seen on the same chromatogram as the amino acids. No red colour was formed with ferric chloride, which indicates that simple aminohydroxamic acids were not in question. It was generally found that in the position for bound hydroxylamine a faint yellow colour was to be seen before ninhydrin spraying.

The bound hydroxylamine found in the culture solutions of *Azotobacter* cells grown with different nitrogen nutrition (N_2, NO_3^-, NH_4^+) behaved in much the same way on ion exchangers. On reduction with sodium amalgam amino acids were not formed from the bound hydroxylamine which had passed through the Amberlite IR-120 column, and hence the oximes of keto acids were not found in this fraction. This fraction, the quantity of which varied in different experiments generally constituting only a few per cent of the total bound hydroxylamine, was not closer investigated.

It is remarkable that from a one-dimensional paper chromatogram a thorough extraction brought only about 20 % of the bound hydroxylamine originally placed on the paper into water solution and about 30 % into water containing ammonia.

Bound hydroxylamine was freed from amino acids in the cellulose powder column using butanol-acetic acid as solvent. Bound hydroxylamine travelled slowest (*cf.* above), and the yellow colour made it easy to follow its run. It cannot be said for certain if the colour belongs to the hydroxylamine fraction or if it is only an impurity in this fraction. Generally it was found, however, that there was some parallelism between the yellow colour and the content of bound hydroxylamine in *Azotobacter* cultures.

On hydrolysis with 6 N HCl two unknown amino acids were formed from the bound hydroxylamine fraction and in addition glutamic acid and smaller amounts of glycine, serine, alanine, valine, and *iso*leucine. On a two-dimensional paper chromatogram the unknown amino acids travelled slowly both in phenol and butanol-acetic acid and formed one spot, provided that the run with butanol-acetic acid was not continued for 4 to 5 days. In the latter case the spot divided into two. The faster travelling spot was chromatographically identical with synthetic hydroxyaspartic acid (R_F 0.05 in both solvents). When this spot was cut off from the paper and the water extract reduced with HI and red phosphorus, mainly aspartic acid, but also small amounts of glycine, serine and alanine were formed, *i. e.* the same products as from syn-

thetic hydroxyaspartic acid. On the basis both of chromatographic behaviour and reduction products it can be regarded as proved that the amino acid studied is hydroxyaspartic acid.

The spot which travelled slower in butanol-acetic acid (R_F ~0.04) gave on reduction with HI and phosphorus glutamic acid and in addition glycine, serine, and alanine. It may be dihydroxyglutamic acid, but this cannot be regarded as proved.

Because there was no proof of the homogeneity of the isolated bound hydroxylamine fraction, it was subjected to paper electrophoresis (pyridine-acetic acid buffer, 1 000 V, 10 mA, pH 6.5, 4 h). Upon this bound hydroxylamine divided into two fractions, one of which behaved neutral and the other slightly basic (Fig. 2). On hydrolysis the neutral fraction gave hydroxyaspartic acid, glutamic acid, and alanine (in addition traces of valine and leucine), the basic fraction again the other unknown amino acids and besides the same amino acids as the neutral one. From the results it can be concluded that there are two hydroxylamine compounds, one giving hydroxyaspartic acid on hydrolysis, the other a still slower in butanol-acetic acid moving acidic amino acid (*cf.* above). If the components separated by electrophoresis are homogeneous they may be polypeptides, in which hydroxylamine is perhaps bound to one amino acid. The small amount of hydroxylamine formed on hydrolysis of these compounds — only about 1.5 % of the total N — arouses, however, the suspicion that the electrophoretically separated fractions may contain peptides as foreign substances. If this is the case nothing can be said about the chemical nature of the hydroxylamine compounds. Attention should, however, be called to the fact that hydroxyaspartic acid and another not fully characterized acid amino acid apparently belong to bound hydroxylamine in some way, since in *Azotobacter* cultures in which bound hydroxylamine was not formed, these amino acids were not found either.

A further elucidation of the problem demands more material and, accordingly, cultivation of *Azotobacter* on a larger scale. In any case it was proved that structurally complicated hydroxylamine compounds are accumulated in the culture solution of *Azotobacter;* that these compounds can be separated from amino acids by chromatography, and from each other by electrophoresis; that hydroxyaspartic acid is formed from one of these compounds on total hydrolysis and another not fully characterized amino acid from the other. In addition to these glutamic acid and alanine were formed from both.

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X-Ray Crystallographic Data on Miscellaneous Sulphur Compounds

OLAV FOSS

Chemical Institute, University of Bergen, Bergen, Norway

The unit cells and space groups of the following derivatives of divalent sulphur, selenium and tellurium were determined some years ago in the course of crystal structure work on analogous compounds, and have not been published before. The data were derived from oscillation and Weissenberg photographs on single-crystal specimens, using iron radiation, $\lambda(\mathrm{Fe}K\alpha) = 1.934$ Å, and in one case (tellurium dibenzenethiosulphonate) copper radiation, $\lambda(\mathrm{Cu}K\alpha) = 1.542$ Å.

Tellurium dimethylxanthate [1], $\mathrm{Te(S_2C\text{-}OCH_3)_2}$. Monoclinic prismatic, $a = 4.24$ Å, $b = 14.18$ Å, $c = 17.28$ Å, $\beta = 93°$. There are four molecules per unit cell; density, calc. 2.19, found 2.18 g/cm³. The space group, from systematic absences, is $C_{2h}^5 - P2_1/c$.

The crystals occur as brownish red prisms, extended along [100] and with {001} dominant. The repeat distance along the a axis, 4.24 Å, is shorter than twice the value, 2.2 Å, listed by Pauling [2] for the van der Waals radius of tellurium, but is not in discord with Briegleb's value [3], 1.9 Å, or with the non-bonded Te-Te approaches of 4.18 Å and 4.28 Å, respectively, found in *p,p'*-dichlorodiphenyl ditelluride [4] and monoclinic barium telluropentathionate dihydrate [5].

The crystals liberate tellurium more rapidly than do those of the ethyl compound described below, perhaps because of the close Te-Te contact.

Tellurium diethylxanthate [1], $\mathrm{Te(S_2C\text{-}OC_2H_5)_2}$. Monoclinic prismatic, $a = 9.35$ Å, $b = 6.17$ Å, $c = 21.21$ Å, $\beta = 91°$. Four