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

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

Since hydroxyamine 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.


Received September 22, 1957.

On Hydroxyamine Compounds in *Azotobacter* Cultures. II. On the Chemical Nature of the Bound Hydroxyamine 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 hydroxyamine was accumulated in the nutrient solution of *Azotobacter*. Attempts to isolate and characterize these hydroxyamine compounds are reported in this paper.

**Behaviour on ion exchange columns.** Bound hydroxyamine 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 hydroxyamine passed through the Amberlite IR-120 column. From this column the amino acids and part of the hydroxyamine compounds could be eluted by 1 N ammonia, the latter compounds quantitatively only with 10% ammonia. Paper chromatographic characteristics showed that the bound hydroxyamine eluted together with amino acids by dilute ammonia was similar to that eluted by more concentrated ammonia.

**Separation of the bound hydroxyamine 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 hydroxyamine (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 hydroxyamine (a weak greyish spot with ninhydrin), 1 = gly, 2 = ala, 3 = val, 7 = tyr, 8 = ser, 14 = arg, 15 = lys, 16 = aep, 17 = glu.

*Acta Chem. Scand.* 11 (1957) No. 8
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 \( \beta \)-aspar-tylhydroxamic and \( \gamma \)-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 aminohydrox- amic 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 con-
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 (Rf ~ 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 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 addi-

tion to these glutamic acid and alanine were formed from both.


Received September 22, 1957.

X-Ray Crystallographic Data on Miscellaneous Sulphur Compounds

Olaav 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, \( \lambda(FeK) = 1.934 \, \text{A} \), and in one case (tellurium dibenzethiosulphonate) copper radiation, \( \lambda(CuK) = 1.542 \, \text{A} \).

Tellurium dimethylxanthatate\(^1\), \( \text{Te(S)}_2\text{C}(-\text{OCH})_2\text{H}_2 \). Monoclinic prismatic, \( a = 4.24 \, \text{A} \), \( b = 14.18 \, \text{A} \), \( c = 17.28 \, \text{A} \), \( \beta = 93^\circ \). There are four molecules per unit cell; density, calc. 2.19, found 2.18 g/cm\(^3\). The space group, from systematic absences, is \( C_{1h}^3 = P2_1/c \).

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

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

Tellurium diethylxanthatate\(^1\), \( \text{Te(S)}_2\text{C}(-\text{OC}_{2}\text{H}_{5})_2 \). Monoclinic prismatic, \( a = 9.35 \, \text{A} \), \( b = 6.17 \, \text{A} \), \( c = 21.21 \, \text{A} \), \( \beta = 91^\circ \). Four

Acta Chem. Scand. 11 (1957) No. 8