when h resp. k is odd, suggesting that the space group may be $V^3-P2_12_12$, but absences cannot be stated with certainty from powder patterns alone, and the crystals were too small for single crystal methods.

A powder photograph of mannitol from alcohol-aceton-water is identical with that of mannitol prepared from the triethylidene compound (see Meunier 4). Purified by the latter method, followed by slow crystallization from 96 % alcohol, good crystals for X-ray investigations were obtained. Unit cell dimensions of the orthorhombic crystals were determined from oscillation and Weissenberg photographs: a = 8.94 A, b = 18.41 A, c =4.92 A, a:b:c = 0.486:1:0.267, V = 809.7A³, Z = 4 and $d_x = 1.494$ g/cm³. Many absences are recorded, thus, h00 when hodd, 0k0 when k odd, 00l when l odd, 0klwhen l odd, h0l when h+l odd and hk0when h odd. No orthorhombic space group has all these absences, and some of them must, therefore, be due to pseudosymmetry. By re-exammination of the photographs a few exceedingly weak reflexions 0k0 were found for which k is odd. thus [010] is no true screw axis. The remaining absences would suggest the space group V_h⁴-Pcna, but no reasonable arrangement of the molecules could be found. On the assumption that absences of 0kl for l odd are also due to pseudosymmetry (these absences being less certain since reflexions 0kl for l even are also very weak), the others correspond to the space group C_{2v}^6-Pmna . A satisfactory arrangement of the molecules can be found such that the direction of their carbon chains is parallel to [010] and their approximate centers are located close to the positions $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ $\frac{1}{4}$ This arrangement would also account for the systematic weaknesses.

The axial ratio of this form agrees roughly with that quoted by Groth for α -mannitol (with c-axis doubled), and this

form may, therefore, possibly correspond to α -mannitol.

- Groth, P. Chemische Krystallographie 3 (1910) 431. Leipzig.
- Becker, K. and Rose, H. Z. Phys. 14 (1923) 369.
- Marwick, Th. C. Proc. Roy. Soc. A 131 (1931) 621.
- 4. Meunier, J. Compt. Rend. 108 (1889) 408.

Received September 4, 1952.

The Presence of Djencolic Acid in Hydrolysates of Mammalian Tissues

GUNNAR ÅGREN and STEN EKLUND

Department of Medical Chemistry, University of Uppsala, Sweden

Diencolic acid was first isolated from urine of natives of Java who had eaten the djencolic bean and were suffering from djencolic poisoning. Later on Van Veen and Hyman isolated the compound from alkaline extracts of the bean ^{1,2}. Consden et al.³ found the amino acid in hydrolysates of reduced wool treated with methylene bromide or formaldehyde. To our knowledge the amino acid has not been isolated from common protein hydrolysates of mammalian tissues.

In connection with microbiological and chemical analysis of a peptide fraction isolated from calf plasma ⁴ we found that the two-dimensional chromatograms of the acid-hydrolyzed material (3 N HCl, 16 hours at 120°) showed a spot close to the known positions of ethanolamine-phosphoric acid, djencolic acid and diaminopimelic acid ^{5–7}.

The substance was eluted with HCl together with the leucine-isoleucine fraction from a Dowex 50 columns according to the method of Stein and Moore 8. The dry mixture was packed on a small column and eluted with tert. — amyl alcohol where

djencolic acid was insoluble. The amino acid was recrystallized three times from 6 N HCl in the form of needles. They were compared with djencolic acid synthesised according to du Vigneaud and Patterson 9 . They had the same R_F -values in paper chromatograms run with different solvents. The X-ray powder diagrams were the same. The molecular weight was 325 as calculated from X-ray diffraction data, suggesting the presence of four molecules per unit cell. The substance had the following composition.

Calc. C 25.6 H 4.9 S 19.6 Cl 22.3 N 8.6 Found » 23.4 » 4.5 » 20.2 » 23.0 » 9.0

Scarcity of material allowed only a single analysis. The results seem to indicate that djencolic acid is present in the plasma peptide fraction.

A spot corresponding to the position of djencolic acid has also been observed in chromatograms fromacid-hydrolyzed plasma, liver and muscle tissues. With regard to the quantitative aspects the chromatograms showed the following pictures when amounts of material corresponding to $100 \mu g$ of material were used. The strongest spot was obtained from the peptide fraction. Liver and plasma contained smaller amounts of the amino acid and in muscle hydrolysates the spot was barely visible. Negative results were obtained with hydrolysates of proteins from the spleen and with a peptide fraction prepared from human urine. On the other hand, recent results in this laboratory with chromatograms of some mucoproteins seem to show the presence of a spot close to the position of diencolic acid 10.

Accordingly, our results are in good agreement with the opinion expressed already in 1936 by du Vigneaud 9: "It is possible that the compound may be more widely distributed in nature and perhaps may be responsible for a portion of the non-cystine, non-methionine sulfur in certain protein." A full account of this work will appear elsewhere.

The investigation was supported by a grant from the Medical Research Council. The technical assistance of Mr. T. Persson is greatfully acknowledged.

- Van Veen, A. G., and Hyman, A. J. Geneesk, Tijdschr. Nederland. Indië 73 (1933) 991.
- Van Veen, A. G., and Hyman, A. J. Rec. trav. chim. 54 (1935) 493.
- Consden, R., Gordon, A. H., and Martin, A. J. P. Biochem. J. 40 (1946) 580.
- 4. Ågren, G. Acta Chem. Scand. 6 (1952). In the press.
- 5. Dent, C. E. Biochem. J. 43 (1948) 169.
- Ågren, G., and Nilsson, T. Acta Chem. Scand. 3 (1949) 525.
- Work, E. Biochem. et Biophys. Acta 5 (1950) 204.
- Stein, W. H., and Moore, S. Cold Spring Harbor Symposia Quant. Biol. 14 (1950) 179
- du Vigneaud, V., and Patterson, W. I. J. Biol. Chem. 114 (1936) 533.
- 10. Werner, I. Personal communication.

Received September 26, 1952.

Separation of Growth Factors for Lactobacillus lactis Dorner Lactobacillus leichmannii and Leuconostoc citrovorum, by Means of Ionophoresis on Paper

L.-E. ERICSON, Z. G. BÁNHIDI and G. GASPARETTO

Division of Food Chemistry, Royal Institute of Technology, Stockholm, Sweden

The usefulness of microorganisms for identifying and estimating biologically active substances is in some cases limited, because of the lack of specificity. In the