

However, some other experiments have shown that the steric factor is not the only important one in nitrations. Under the same experimental conditions that applied in the nitration of 1,3-xylene, we found that toluene and chlorobenzene yielded decreasing proportions of the 2-nitro isomers as the pressure was increased. The results are given in Table 2.

Table 2. MOLE FRACTIONS OF ISOMERS FORMED IN THE NITRATION OF TOLUENE AND CHLOROBENZENE BY NITRIC ACID IN ACETIC ACID SOLUTION AT 0° C

| Pressure (atm.) | Toluene       |       |       | Chlorobenzene |    |       |
|-----------------|---------------|-------|-------|---------------|----|-------|
|                 | Nitro isomer: |       |       | Nitro isomer: |    |       |
|                 | 2-            | 3-    | 4-    | 2-            | 3- | 4-    |
| 1               | 0.560         | 0.020 | 0.420 | 0.247         | —  | 0.753 |
| 1,200           | 0.550         | 0.022 | 0.428 |               |    |       |
| 2,000           | 0.543         | 0.024 | 0.433 | 0.204         | —  | 0.796 |

Although the shift of isomer ratio in these cases was relatively small, there was a consistent trend with increasing pressure and its direction was the same whether the groups already present on the benzene ring had electron donating (activating) or electron accepting (de-activating) properties.

Experiments at atmospheric pressure<sup>5</sup> have established that a change of solvent alters the ratios of isomers formed in the nitration of alkyl benzenes by nitronium salts. The effect almost certainly arises from changes of dielectric constant and solvation. Presumably similar factors operate when the dielectric properties of the nitrating solution are altered by an applied pressure, and they are supplementary to the simple steric effect. Moreover, it is possible that compression may alter the distribution of electrical charge around the benzene rings. We hope to distinguish these factors more clearly in future work.

D. W. COILLET\*  
S. D. HAMANN

C.S.I.R.O.

Division of Physical Chemistry,  
Melbourne.

\* Present address: Department of Chemical Engineering, University of Sydney.

<sup>1</sup> Gonikberg, M. G., Prokhorova, N. I., and Litvin, E., *Izv. Akad. Nauk. S.S.S.R. (Otd. Khim. Nauk.)*, No. 8, 1495 (1962).

<sup>2</sup> Gonikberg, M. G., Prokhorova, N. I., and Litvin, E., *Doklady Akad. Nauk. S.S.S.R.*, **148**, 105 (1963).

<sup>3</sup> Gonikberg, M. G., *Zhur. Fiz. Khim.*, **37**, 477 (1963).

<sup>4</sup> Coillet, D. W., and Hamann, S. D., *Trans. Farad. Soc.*, **57**, 2231 (1961).

<sup>5</sup> Olah, G. A., and Kuhn, S. J., *J. Amer. Chem. Soc.*, **84**, 3684 (1962).

## BIOCHEMISTRY

### Amino-acid Sequences within the Ferrichrome Cyclic Hexapeptides

An earlier communication<sup>1</sup> concerned with the structures of ferrichrome and ferrichrome A left open the particular arrangement of the 3 residues of neutral amino-acids and 3 residues of  $\delta$ -N-hydroxyornithine<sup>2</sup> within the cyclic hexapeptide moiety of the naturally occurring ferric trihydroxamates. The peptide portion of ferrichrome contains 3 residues each of glycine and  $\delta$ -N-hydroxyornithine while, in ferrichrome A, 2 of the 3 glycine residues are replaced by serine residues. In the present report we propose that the sequence for ferrichrome is *cyclo*-triglycyl-tri- $\delta$ -N-hydroxyornithyl while that for ferrichrome A is most probably *cyclo*-diserylglycyl-tri- $\delta$ -N-hydroxyornithyl.

Ferrichrome was hydrogenated with Raney nickel in order to eliminate both the iron and the three oxygen atoms of the hydroxamate functions<sup>3</sup>. The product was precipitated from methanol-acetone to yield colourless prisms with m.p. 262°–265° (uncorr., decomp.). A synthetic specimen was obtained by the successive addition of three residues of  $\alpha$ -N-carbo-benzyloxy- $\delta$ -acetylornithine-p-

nitrophenyl ester to triglycine ethyl ester, followed by de-blocking and cyclization of the linear hexapeptide. This choice of sequence was predicted on the observation that diglycine appears during partial acid hydrolysis of ferrichrome and on the recent discovery by Czech workers<sup>4</sup> that triserine occurs in the related compound albomycin. The natural and synthetic products displayed the same m.p. (decomp.) and the same  $R_F$  in acidic, neutral and basic solvents. Exposure of the two preparations to 12 N hydrochloric acid at 30° for 1.5 days provided an identical array of ninhydrin-positive spots after analysis by paper electrophoresis in 4 per cent formic acid.

Ferrichrome A was degraded by a bacterium which had been isolated from soil through enrichment culture on the ferrichrome compounds as sole source of carbon and nitrogen. The complex pattern of peptides present in the culture medium was separated by Moore-Stein<sup>5</sup> chromatography and selected members were subjected to sequence analysis by use of standard reagents such as fluorodinitrobenzene and hydrazine. These investigations revealed the presence of the tripeptide seryl-glycyl- $\delta$ -N-acylhydroxyornithine. A dipeptide, believed to be serylserine, was produced through partial acid hydrolysis of iron-free ferrichrome A.

In summary, it appears that the cyclic hexapeptide core of the ferrichrome growth factors shares a common component with the albomycin antibiotics, namely, a tripeptide of  $\delta$ -N-hydroxyornithine.

This work was supported by the U.S. Public Health Service (grant No. E-4156) and the Office of Naval Research (grant No. 222-39). The Moore-Stein chromatography was performed through the courtesy of Dr. R. David Cole.

S. J. ROGERS  
R. A. J. WARREN  
J. B. NEILANDS

Department of Biochemistry,  
University of California,  
Berkeley.

<sup>1</sup> Emery, T., and Neilands, J. B., *J. Amer. Chem. Soc.*, **83**, 1626 (1961).

<sup>2</sup> Rogers, S., and Neilands, J. B., *Biochemistry*, **2**, 6 (1963).

<sup>3</sup> Gipson, R. M., Pettit, F. H., Skinner, C. G., and Shive, W., *J. Org. Chem.*, **28**, 1425 (1963).

<sup>4</sup> Mikeš, O., and Turková, J. (personal communication).

<sup>5</sup> Spaekman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).

### Hydrolysis of Some Antibiotics and of their Decomposition Products in the Presence of Cation Exchange Resins

THE conditions under which some antibiotics are hydrolysed may be important for the isolation and identification of the products of their decomposition since these are used in investigations of the structure of the antibiotics. During hydrolysis with mineral acids some moieties, such as carbohydrates, may be destroyed, and much humin may be produced which makes it difficult to isolate the more stable products of decomposition in pure form.

A new method of hydrolysis of some antibiotics with the aid of sulphocationic resins of the 'Dowex-50' type is described here. By varying the conditions of the experiments (temperature, time, type of resin) different products of hydrolysis may be obtained without their complete degradation and without the production of humins. At the same time basic substances can be separated from neutral and acid products. The basic substances are adsorbed on the cationic resin while the neutral and acid products are retained in the mother liquor.

By gradient elution with various eluants different basic products adsorbed on the ion-exchange resins can be separated. The following sulphonic resins were used for hydrolysis: 'Dowex-50 × 12' (50–100 and 100–200 mesh)