Fig. 2. α is plotted against β . $\circ$ denotes values obtained by titrations. ($\times$) denotes values obtained by the batch method.

the batch method. The titration method has proved to be much simpler and faster than the batch equilibrium method.

It may be noted that only about half the β interval has, in this case, been covered by the titration method. By starting from Ag^+ saturated resin and AgNO_3 it is possible to cover the whole interval by titrations. However, the amount of H^+ in the solution is, in this case, very uncertain as it is determined as a difference. In this case it would be better to use a H^+ sensitive electrode e.g. a glass electrode and change to a Ag/AgI electrode for $\beta \approx 0.5$.

As electrodes sensitive to a number of ions are available, many ion exchange equilibrium studies can be carried out as emf titrations. For instance the $\text{Cu}^{2+} - \text{H}^+$ exchange can be studied with both a copper amalgam and a glass electrode. The diffusion potentials must be known for the ion

pair and the ionic strength in question. They can according to Ekedahl⁵⁻⁶ be determined rapidly by titrations without resin.

1. Duncan, J. F., and Lister, B. A. *J. Chem. Soc.* (1949) 3285.
2. Glueckauf, E. *J. Chem. Soc.* (1949) 3280.
3. Sillén, L. G. *Arkiv Kemi* **2** (1950) 477, 499.
4. Högfeldt, E., Ekedahl, E., and Sillén, L. G. *Acta Chem. Scand.* **4** (1950) 1471.
5. Sillén, L. G., and Ekedahl, E. *Arkiv Kemi, Mineral. Geol.* **A 22** (1946) no. 16.
6. Ekedahl, E., and Sillén, L. G. *Arkiv Kemi, Mineral. Geol.* **A 25** (1947) no. 4.

Received February 20, 1952.

Preparation of D-Valine by Asymmetric, Enzymatic Hydrolysis of the Isobutyl Ester of DL-Valine

K. ARVID J. WRETLIND

Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden

DL-valine has earlier been resolved by fractional crystallization of the brucine salt of its formyl derivative¹ or of its menthoxyacetyl derivative². Duschinsky and Jeannerat³ prepared the L-isomer of valine by oxidation of the D-isomer in the racemic form of valine with D-amino acid oxidase from hog kidneys. Because the enzymes in yeast destroy the L-valine and leave the D-form intact, it is possible with this procedure to obtain the latter free from the L-isomer⁴. Price, Gilbert and Greenstein⁵ produced the valine isomers by means of asymmetric hydrolysis of chloroacetyl-DL-valine.

A simple method for producing D-valine is described in the present paper. It is based on the asymmetric hydrolysis of the isobutyl ester of DL-valine with an enzyme preparation obtained from pancreas. Complete digestion of the ester results in free L-valine and the isobutyl ester of D-valine.

The latter is isolated by means of its solubility in ether and alcohol. The D-valine is obtained after acid hydrolysis of the ester.

It is not, however, possible to isolate L-valine with this technique. This is because the enzymatic hydrolysis of the isobutyl ester of DL-valine is extremely slow, necessitating large quantities of enzyme. This results in an admixture of other amino acids freed from the enzyme preparation, a fact which makes it difficult to isolate L-valine. Attempts were made to do so with this method, both with the help of the formyl derivative and of the esters after distillation, but it proved impossible to obtain pure L-valine. The reason is presumably to be sought in the difficulty, pointed out by Ehrlich and Wendel⁶, in separating isoleucine and valine.

Both the isopropyl ester and the *n*-propyl ester of DL-valine are hydrolyzed asymmetrically with pancreatic enzymes. The isobutyl ester has, however, been found to be more suitable for the preparation of D-valine, since it is more stable in aqueous solution than the *n*-propyl ester and is hydrolyzed more rapidly with the enzyme than the isopropyl ester. Amino acids have earlier been resolved by asymmetric hydrolysis of their esters in order to obtain the isomers of leucine⁷, methionine^{8,9}, phenylalanine¹⁰ and tryptophane¹¹.

Experimental. Isobutyl ester of DL-valine. — 500 g of DL-valine are suspended in 2 500 ml of anhydrous isobutyl alcohol containing 10 per cent of anhydrous hydrochloric acid. The mixture is refluxed for three hours, after which a clear solution is obtained. The solution is evaporated *in vacuo* to an oil. This is mixed with 2 500 ml of anhydrous isobutyl ester containing 10 per cent of hydrochloric acid. After evaporation, the treatment with isobutyl alcohol and hydrochloric acid is repeated. The oil thus obtained is then mixed with 1 000 ml of ether and 500 ml of water and the mixture cooled to -10°C . Concentrated ammonia is added with stirring until the reaction is faintly alkaline to phenolphthalein. The ether layer is separated and the aqueous solution

Fig. 1. Asymmetric hydrolysis of the isobutyl ester of DL-valine with a pancreatic enzyme preparation.

The experiments were made with 1 ml of the isobutyl ester of DL-valine and 5 ml of a 4 per cent pancreatic enzyme solution. The reaction was carried out at room temperature (22°C). Hydrolysis was followed by formol titration. The values given in the curve were obtained after the hydrolysis which occurs when 1 ml of the isopropyl ester is mixed with 5 ml of distilled water had been subtracted from the experimental values. Under these conditions, hydrolysis of the isobutyl ester of DL-valine with water amounted to 4.0 per cent after 24 hours, 7.1 per cent after 48 hours and 9.1 per cent after 72 hours.

extracted twice more, each time with 1 000 ml of ether. The ether extracts are dried over sodium sulfate. The sodium sulfate is removed and washed with 100 ml of anhydrous ether. The ether extracts are distilled *in vacuo* and the fraction distilling between $73-77^{\circ}\text{C}$ at 5 mm contains the isobutyl ester of DL-valine; yield 610–650 g (83 to 88 per cent of theory). $\text{C}_9\text{H}_{19}\text{O}_2\text{N}$ Calculated, N 8.09%; found, N 8.12%.

Enzyme preparation. — The enzymes used in the present investigation were produced as follows. — 180 g of pancreas dry powder according to Willstätter and Waldschmidt-Leitz¹² are shaken for two hours with 1 800 ml of glycerol. The mixture is filtered after the addition of 1 800 ml of water and to the

clear solution two volumes of acetone are added. The precipitate is filtered and the precipitate is washed with 200 ml of acetone and 200 ml of ether. Yield 30–40 g.

The enzyme preparation is dissolved by shaking for 30 minutes in distilled water immediately before use. The rate of hydrolysis is shown in Fig. 1. It is evident from the figure that the D-ester is stable against the pancreatic enzymes for at least 72 hours and that, with the amount of enzymes used, practically all the L-ester is hydrolyzed after 24 hours. The fact that hydrolysis with enzymes is asymmetrical is shown by the method for the preparation of D-valine described in the following. The investigations showed that, under similar experimental conditions, the isobutyl ester of DL-valine is also hydrolyzed to some extent with water.

Preparation of D-valine. — 100 g of the isobutyl ester of DL-valine are shaken for 48 hours with 400 ml of a 5 per cent solution of pancreatic enzymes at room temperature. 400 ml of ether and 20 ml of concentrated ammonia are added to the mixture. The ether layer is separated and the aqueous solution extracted twice more, each time with 100 ml of ether. The ether extracts are dried over sodium sulfate and the ether evaporated after the sodium sulfate has been removed. The remaining oil is shaken for 48 hours at room temperature with 100 ml of 5 per cent pancreatic enzyme solution. While stirring 100 ml of ether and 5 ml of concentrated ammonia are added to the digestion mixture. The ether layer is separated and the aqueous solution extracted twice more, each time with 100 ml of ether. The ether extracts are dried over sodium sulfate. The sodium sulfate is removed and the ether is evaporated. The oil of the isobutyl ester of D-valine thus obtained is refluxed for 12 hours with 350 ml of 20 per cent hydrochloric acid. The solution is evaporated *in vacuo* to dryness and for a second time after adding 100 ml of water. The hydrochloride of D-valine thus obtained is dissolved in 200 ml of 96 per cent alcohol. 50 ml of pyridine are added and the mixture is placed overnight in the refrigerator. The precipitate is filtered off and washed with 50 ml of alcohol and 50 ml of ether. The impure D-valine is recrystallized by dissolving in hot water (11 ml per g), decolorized with 0.5 g of norit and the clear solution treated with an equal volume of 95 per cent alcohol. The precipitate is washed with absolute alcohol and ether as described. To obtain an analytically pure sample, the D-valine is again recrystallized and washed as outlined above. The product thus obtained weighs 14–16 g (41 to 47 per

cent of theory). Analysis of the D-valine obtained with this method gave the following results:

$C_5H_{11}O_2N$ Calculated, N 11.96; found, N 11.95
 $\alpha_D^{23} = -28.2$ to -28.6° ($c = 2$, in 6 N hydrochloric acid).

The figures given in the literature are -29.0° (Fischer¹), -27.1° (Price, Gilbert and Greenstein⁵) and -27.6° (Ehrlich and Wendel⁶) respectively.

1. Fischer, E. *Ber.* **39** (1906) 2320.
2. Holmes, D. F., and Adams, R. J. *Am. Chem. Soc.* **56** (1934) 2093.
3. Duschinsky, R., and Jeannerat, J. *Compt. rend.* **208** (1939) 1359.
4. Ehrlich, F. *Biochem. Z.* **1** (1906) 8.
5. Price, V. E., Gilbert, J. B., and Greenstein, J. P. J. *Biol. Chem.* **179** (1949) 1169.
6. Ehrlich, F., and Wendel, A. *Biochem. Z.* **8** (1908) 399.
7. Warburg, O. *Z. physiol. Chem.* **48** (1906) 205.
8. Brenner, M., and Kocher, V. *Helv. Chim. Acta* **32** (1949) 333.
9. Wretling, K. A. J. *Acta Physiol. Scand.* **20** (1950) 1.
10. Wretling, K. A. J. *J. Biol. Chem.* **186** (1950) 221.
11. Brenner, M., Sailer, E., and Kocher, E. *Helv. Chim. Acta* **31** (1948) 1908.
12. Waldschmidt-Leitz, E. *Z. physiol. Chem.* **132** (1923) 181.
12. Willstätter, R., and Waldschmidt-Leitz, E. *Z. physiol. Chem.* **125** (1923) 150.

Received March 15, 1952.

On the Alleged Complexity of Sodium in Polyhexametaphosphate

BERNHARD ANDERSEN and
KIRSTEN LEHMANN

Laboratory of Zoophysiology, University of
Copenhagen, Copenhagen, Denmark

According to Lamm and Malmgren¹ the Na in $K_2Na_4(PO_3)_6$ is bound in complex form. That is, they state that they have dialyzed a solution of the polyhexametaphosphate (PMP) against 0.1 N HCl, and found that only K exchanges with