An Obstacle in Preparing Alloisoleucine

By W. M. LAIRD, S. MATAI* AND R. L. M. SYNGE

*Agricultural Research Council Food Research Institute,
Colney Lane, Norwich NOR 70F, Norfolk, U.K.*

(Received 13 October 1969)

Alloisoleucine, prepared from a mixture of racemic isoleucine-alloisoleucine diastereoisomers, can be heavily contaminated with isoleucine if the recrystallization of acetyl-DL-alloisoleucine is controlled by melting point only. Two commercial specimens were found to be thus contaminated.

We have recently had to prepare derivatives of alloisoleucine, starting from a commercial racemic mixture of the four isoleucine-alloisoleucine diastereoisomers. To segregate the alloisoleucine we followed the procedures of Greenstein and colleagues (Greenstein, Levintow, Baker & White, 1951; Greenstein, Birnbaum & Levintow, 1953; Greenstein & Winitz, 1961), but found that acetyl-DL-alloisoleucine once recrystallized from aq. 50% (v/v) acetic acid and having a melting point (as recommended) above 167°C could have an alloisoleucine/isoleucine ratio as low as 10. Three recrystallizations were needed to increase the ratio to 20 and a further two to bring it to over 100. These ratios were determined by acid hydrolysis of the acetyl derivatives followed by chromatography of the resulting amino acids on sulphonated polystyrene resin, as described in the Experimental section (cf. Piez, 1954; Moore, Spackman & Stein, 1958; Hamilton, 1963).

Moore *et al.* (1958) reported that, under the usual conditions for hydrolysis of proteins (refluxing in 6M-HCl for 24 h), approx. 3% of isoleucine became epimerized at the α -carbon atom. As our conditions of hydrolysis were much less severe, it seems probable that the isoleucine found (<1%) in our best product was due to residual impurity of the acetyl-DL-alloisoleucine, rather than to epimerization during hydrolysis.

In the course of the recrystallizations, the melting point first rose to 169–170°C (corr.) but later fell to 167°C (corr.). We conclude that the melting point is of little value as a criterion of purity for acetyl-DL-alloisoleucine.

We found commercial preparations of acetyl-D-alloisoleucine (lot no. A60-92; Sigma Chemical Co.,

St Louis, Mo., U.S.A.) and of D-alloisoleucine (A grade, lot no. 671422; Calbiochem, Los Angeles, Calif., U.S.A.) to have alloisoleucine/isoleucine ratios of 25 and 20 respectively.

EXPERIMENTAL

Starting material was 'DL-isoleucine (mixture of four stereoisomers)' (lot no. 27B-0120; Sigma), which we found to have an alloisoleucine/isoleucine ratio of 1:13.

Recrystallizations were done by dissolving the material in 4–5 parts (w/w) of hot aq. 50% (v/v) acetic acid, filtering off after keeping at 1°C overnight and washing the crystals with a little water. Acetyl derivatives were hydrolysed for amino acid analysis by refluxing for 2 h in 2M-HCl followed by evaporation to dryness *in vacuo*. For chromatography the Beckman-Spinco Amino Acid Analyzer (model 120C) was used with column dimensions 56 cm $\times$ 0.9 cm, successive development with the recommended pH 3.25 and pH 4.3 buffers and column temperature 55°C.

We are grateful to Miss Janice Payne for help with the preparation and to Mrs Julia Levett for the chromatographic analyses. S.M. acknowledges maintenance allowances under the Colombo Plan from the U.K. Ministry of Overseas Development and the Government of India.

REFERENCES

- Greenstein, J. P., Birnbaum, S. M. & Levintow, L. (1953). *Biochem. Prep.* **3**, 84.
- Greenstein, J. P., Levintow, L., Baker, C. G. & White, J. (1951). *J. biol. Chem.* **188**, 647.
- Greenstein, J. P. & Winitz, M. (1961). *Chemistry of the Amino Acids*, vol. 3, p. 2043. New York: John Wiley and Sons Inc.
- Hamilton, P. B. (1963). *Analyt. Chem.* **35**, 2055.
- Moore, S., Spackman, D. H. & Stein, W. H. (1958). *Analyt. Chem.* **30**, 1185.
- Piez, K. A. (1954). *J. biol. Chem.* **207**, 77.

* Present address: Indian Statistical Institute, 203 Barrackpore Trunk Road, Calcutta-35, India.