- Folley, S. J. (1952). *Symp. biochem. Soc.* no. 9, p. 52.
 Folley, S. J. & French, T. H. (1950). *Biochem. J.* **46**, 465.
 Green, D. E. & Mii, S. (1953). *Fed. Proc.* **12**, 211.
 Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.
 Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **39**, 442.
 Moore, R. O. & Nelson, W. L. (1952). *Arch. Biochem. Biophys.* **38**, 178.
 Popják, G. (1950). *Biochem. J.* **46**, 560.
 Popják, G. (1952a). *Nutr. Abstr. Rev.* **21**, 535.
 Popják, G. (1952b). *Symp. biochem. Soc.* no. 9, p. 37.
 Popják, G., French, T. H., Hunter, G. D. & Martin, A. J. P. (1951). *Biochem. J.* **48**, 612.
 Popják, G. & Tietz, A. (1953a). *Biochem. J.* **54**, xxxv.
 Popják, G. & Tietz, A. (1953b). *Biochem. J.* **54**, xxxv.
 Stadie, W. C. & Riggs, B. C. (1944). *J. biol. Chem.* **154**, 687.

Paper-chromatographic Analysis of Dakin's Samples of ' β -Hydroxyglutamic Acid'

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(Received 1 May 1953)

Dakin twice claimed the isolation from proteins of β -hydroxyglutamic acid. In his first paper (Dakin, 1918) he obtained a 10.5% yield but later (Dakin, 1919) he reported more variable yields of 2–10%. The compound had been obtained by acid and by tryptic digestion of casein and also by acid hydrolysis of gliadin and glutenin. Many workers have been unable to repeat this work and doubt is now generally cast on its validity (Nicolet & Shinn, 1942; Bailey, Chibnall, Rees & Williams, 1943; Beveridge & Lucas, 1944). It is of note that Dakin himself in a footnote to one of his papers (Dakin, 1941) commented on the fact that one reaction of his amino acid was inconsistent with its structure as a β -hydroxy- α -amino acid and that he was therefore exploring further the possibility that it was a γ -hydroxy compound. The amino acid still, however, continues to occupy space in the standard textbooks, since, using classical methods of analysis and isolation, it is difficult to be certain of the absence from a protein of such a substance. The only certain way to assess the earlier work is to re-examine the original samples in question. Dr H. B. Vickery has now kindly obtained for us two samples of β -hydroxyglutamic acid from Dakin's collection. These we have analysed by the simpler and far more specific methods now available.

MATERIALS

Two of Dakin's specimens were obtained from the Dakin Collection, Adelphi College. They were labelled: (1) 'Hydroxyglutamic acid *ex* casein and trypsin.' (2) 'Hydroxyglutamic acid, analysed.' (1) was a mixture of brown particles and powder, (2) comprised 1 mg. only of brown particles. They will be referred to here as Dakin's specimens nos. 1 and 2, respectively.

For comparison with Dakin's samples, synthetic specimens of DL- β -hydroxyglutamic and DL-*allo*- β -hydroxy-

glutamic acids were obtained by Dr Vickery from Dr Pfister. Both substances were white crystalline powders. The methods of synthesis and the convention of nomenclature have been described by Leanza & Pfister (1953).

METHODS

The analyses were performed on 0.1% (w/v) solutions of the amino acids in 10% (v/v) aqueous isopropanol. The two Dakin specimens dissolved immediately in the solvent, whereas the two synthetic compounds only dissolved slowly after much shaking. The amino acids used as markers were in the same solvent at a concentration of 0.01 M.

One-way paper chromatograms were run with phenol:water in the presence of NH_3 and HCN. Two-way chromatograms were run with this system first followed by collidine:lutidine:water in the presence of diethylamine vapour. The apparatus and working details were as described by Dent (1948). Both the synthetic isomers of β -hydroxyglutamic acid gave brownish purple spots on the chromatogram which could be readily distinguished from the adjacent spots given by other amino acids.

Periodate treatment of the amino acids was carried out by adding about 2 vol. of saturated aqueous KIO_4 and 0.5 vol. of saturated aqueous Na_2CO_3 to 1 vol. of amino acid solution. The mixture was allowed to stand for 0.5 hr. and was then electrolytically desalted in a micromodification (Dent, 1952) of the apparatus of Consden, Gordon & Martin (1947).

RESULTS

Chromatograms of the various samples compared with authentic amino acids were prepared as shown on Pl. 1. They were prepared with the intention of showing whether Dakin's samples contained any β -hydroxyglutamic acids, whether they contained any other β -hydroxyamino acids or any of the commonly occurring amino acids, and if so which. The conclusions drawn from the analyses are written into the description of the Plate (p. 55).

C. E. DENT AND DOROTHY I. FOWLER—PAPER-CHROMATOGRAPHIC ANALYSIS OF DAKIN'S SAMPLES OF ' β -HYDROXYGLUTAMIC ACID'

SUMMARY

1. Two samples of material isolated from protein hydrolysates by the late H. D. Dakin, and alleged by him to be β -hydroxyglutamic acid, consist of mixtures of commonly occurring amino acids, mainly of glutamic and aspartic acids.

2. No β -hydroxyglutamic acid was present in either sample.

We wish to thank Dr H. B. Vickery for his initiative in stimulating this work and for obtaining the samples for us.

REFERENCES

- Bailey, K., Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 366.
 Beveridge, J. M. R. & Lucas, C. C. (1944). *Biochem. J.* **38**, 88.
 Consden, R., Gordon, A. H. & Martin, A. J. P. (1947). *Biochem. J.* **41**, 590.
 Dakin, H. D. (1918). *Biochem. J.* **12**, 290.
 Dakin, H. D. (1919). *Biochem. J.* **13**, 398.
 Dakin, H. D. (1941). *J. biol. Chem.* **140**, 847.
 Dent, C. E. (1948). *Biochem. J.* **43**, 169.
 Dent, C. E. (1952). *Recent Advances in Clinical Pathology*, 2nd ed. Churchill: London.
 Leanza, W. J. & Pfister, K. (1953). *J. biol. Chem.* **201**, 377.
 Nicolet, B. H. & Shinn, L. A. (1942). *J. biol. Chem.* **142**, 139.

EXPLANATION OF PLATE 1

All the chromatograms were run by the descending technique. The one-way chromatograms shown on Figs. 1 and 2 were run with phenol-water: NH_3 :HCN (shown in an upward direction), the amino acids being applied on the baseline as marked. The two-way chromatograms (Figs. 3-8) were run with phenol-water: NH_3 :HCN as first solvent (shown right to left) followed by collidine:lutidine:water:diethylamine (shown in an upward direction); the origin is near the plate number.

The spot identifications are as follows: 1, aspartic acid; 2, glutamic acid; 3, glycine; 4, serine; 5, alanine; 6, leucine and/or isoleucine; 7, threonine; 8, valine; 9, phenylalanine; 10, tyrosine; 11, lysine; 12, β -hydroxyglutamic acid; 13, *allo*- β -hydroxyglutamic acid; 14, taurine.

Fig. 1

One-way chromatogram. Solutions of the following substances were placed on the correspondingly marked starting positions: A, L-glutamic acid (5 $\mu\text{l.}$); B, Dakin's specimen no. 1 (10 $\mu\text{l.}$); C, Dakin's specimen no. 2 (10 $\mu\text{l.}$); D, DL- β -hydroxyglutamic acid (5 $\mu\text{l.}$); E, DL-*allo*- β -hydroxyglutamic acid (5 $\mu\text{l.}$); F, glutamic (5 $\mu\text{l.}$) and β -hydroxyglutamic (5 $\mu\text{l.}$) acids; G, Dakin's specimen no. 1 (10 $\mu\text{l.}$); H, glutamic (5 $\mu\text{l.}$) and *allo*- β -hydroxyglutamic (5 $\mu\text{l.}$) acids; I, Dakin's specimen no. 2 (10 $\mu\text{l.}$); J, taurine (50 $\mu\text{l.}$). The numbers indicate the provisional identifications of the spots. It was concluded that neither of Dakin's specimens contain an amino acid behaving like a β -hydroxyglutamic acid.

Fig. 2

One-way chromatogram. Solutions of the following substances were placed on the correspondingly marked starting positions with additional pretreatment as indicated: A, L-glutamic (5 $\mu\text{l.}$) and DL- β -hydroxyglutamic (5 $\mu\text{l.}$) acids and DL-threonine (5 $\mu\text{l.}$); B, glutamic (15 $\mu\text{l.}$) and β -hydroxyglutamic (15 $\mu\text{l.}$) acids and threonine (15 $\mu\text{l.}$), periodate-treated; C, Dakin's specimen no. 1 (20 $\mu\text{l.}$) and threonine (5 $\mu\text{l.}$); D, Dakin's specimen no. 1 (20 $\mu\text{l.}$) and threonine (5 $\mu\text{l.}$), periodate-treated; E, Dakin's specimen no. 2 (20 $\mu\text{l.}$) and threonine (5 $\mu\text{l.}$); F, Dakin's specimen no. 2 (20 $\mu\text{l.}$) and threonine (5 $\mu\text{l.}$), periodate-treated. The numbers indicate the provisional identifications of the spots. It was concluded that the amino acids in Dakin's specimens which behave chromatographically somewhat like synthetic β -hydroxyglutamic acids are not decomposed by periodate and are therefore not β -hydroxy- α -amino acids. Only one amino acid originally in Dakin's specimen is so decomposed; this is presumably serine.

Fig. 3

Two-way chromatogram of Dakin's specimen no. 2 (50 $\mu\text{g.}$) with taurine (10 $\mu\text{l.}$) added as a marker. The numbers indicate the fairly definite identifications that could now be made. It was concluded that this specimen contains glutamic acid with lesser amounts of other known amino acids.

Fig. 4

As Fig. 3, but with DL- β -hydroxyglutamic acid (10 $\mu\text{l.}$) added. It was concluded that β -hydroxyglutamic acid can be distinguished on our chromatograms from the main amino acids present in Dakin's specimen no. 2.

Fig. 5

Two-way chromatogram of L-aspartic and L-glutamic acids, glycine, DL-serine, DL-alanine and taurine (10 $\mu\text{l.}$ of each). This should be compared with the chromatograms of Dakin's specimens no. 1 (Fig. 8) and no. 2 (Fig. 3).

Fig. 6

Two-way chromatogram of Dakin's specimen no. 2 (50 $\mu\text{g.}$) and the same pure amino acids as for Fig. 5 (10 $\mu\text{l.}$ of each). The pure amino acids run to exactly the same positions as do those in Dakin's specimen no. 2.

Fig. 7

Two-way chromatogram of Dakin's specimen no. 2 (250 $\mu\text{g.}$). Even at this overload the amino acid spots shown appear to correspond with known substances, and no spot is seen in the position occupied by β -hydroxyglutamic acid.

Fig. 8

Two-way chromatogram of Dakin's specimen no. 1 (50 $\mu\text{g.}$) with taurine (10 $\mu\text{l.}$) added. It was concluded that this specimen comprises substantially the same amino acids as no. 2 (Fig. 3) but in different relative proportions.