ci-dessus, et on recristallise le ribitol jusqu'à point de fusion constant (20 mg — F. 99–101° — Activité mesurée en couche mince: 3,200 c.p.m./millimole).

Oxydation périodique du D-*ribitol actif*

On ajoute à une solution de ribitol (17 mg) et de bicarbonate de soude (42 mg) dans l'eau (2 ml) une solution de métapériodate de sodium (106 mg) dans l'eau (2 ml). On laisse une nuit, puis on acidifie (pH 2) avec SO_4H_2 $N/10$ et on ajoute une solution à 20% d'iodure de potassium (1 ml). On ajoute ensuite de l'anhydride arsénieux jusqu'à disparition de l'iode, puis on traite par la soude $N/10$ jusqu'au virage de la phtaléine. Par entraînement à la vapeur, on recueille le formol, précipité par le dimédon (dérivé: 54 mg — 80% — Recristallisé: F. 187–188° — L'activité a été déterminée par un comptage en couche limite: 1,750 c.p.m./milimole).

Le résidu est rendu acide au rouge Congo par SO_4H_2 $N/10$, et l'acide formique distillé avec entraînement à la vapeur. Le distillat est exactement neutralisé à la soude, évaporé à sec et oxydé par l'acétate mercurique (solution à 10% dans l'acide acétique 0.3 N: 10 ml) une demi-heure à chaud dans un appareil à combustion liquide. Le gaz carbonique est recueilli dans la baryte, et le carbonate de baryum centrifugé et lavé (48.5 mg — 73% — Pas d'activité décelable par comptage en couche limite).

L'acide formique correspond aux carbones 2, 3, 4 du D-ribose. Donc ceux-ci sont inactifs. Le formol provient des carbones 1 et 5 et le carbone 5 étant inactif, son activité ne peut être due qu'à la contribution du carbone 1. Dans ces conditions l'activité moléculaire du formal-dimédon doit être la moitié de celle du ribitol. C'est ce que l'on constate, à la précision des mesures.

Ces résultats montrent que le squelette carboné du D-ribose se forme par élimination du carbone aldéhydique du D-glucose.

S. DAVID
J. RENAUT

Laboratoire de Chimie biologique de la Faculté des Sciences, Nancy (France)

[1] S. DAVID ET J. RENAUT, *Bull. soc. chim. biol.*, 36 (1954) 1311.

Reçu le 26 janvier 1955

The application of Edman's peptide degradation method to horse myoglobin and haemoglobin

A number of attempts has recently been made to degrade certain peptides and proteins in a stepwise manner by the phenyl*iso*thiocyanate procedure of EDMAN[1]. In the case of insulin[2] the expected amino-acids were liberated from the two chains for a few steps, but soon complex mixtures were obtained and the sequence was no longer clear. FRAENKEL-CONRAT[3] applied a micro-modification of this procedure to a number of proteins and found that the method gave unambiguous results only for the first 4–5 amino acids.

Horse myoglobin has one N-terminal amino acid group, glycine[4], and it seemed worth while to apply EDMAN'S method to this protein to see how far it could be degraded. FRAENKEL-CONRAT[3] with his micro method has obtained results with myoglobin (presumably from horse) which are in agreement with those reported here.

In connection with X-ray studies in this laboratory on horse haemoglobin, it became desirable to confirm by the EDMAN method that the protein has six valines as N-terminal groups[4], and this was found to be the case.

The method for preparing horse myoglobin was based on that described by SCHMID[5] for whale myoglobin. Instead of treating the crude myoglobin extract with lead acetate to remove non-haeme proteins, it was given two fractionations with ammonium sulphate between 65% and full saturation and afterwards between 75% and full saturation. The protein was crystallised four times from 3 M phosphate buffer pH 6.3 to which solid phosphate buffer[5] had been added. Horse haemoglobin was prepared as described previously[6] and the haeme was removed from the two proteins by the method of ANSON AND MIRSKY[7].

The reaction between the proteins and phenyl*iso*thiocyanate was carried out at pH 9 in aqueous pyridine (1:1) and at room temperature. As reported by other workers the uptake of NaOH never ceased completely, but the reaction was discontinued when the rate of alkali consumption became very slow. Addition of acetone precipitated the phenylthiocarbamyl-(PTC-)-protein and this was extracted with ether. The N-terminal amino acid was split off as its phenylthiohydantoin by allowing a solution of the PTC-protein in anhydrous formic acid to stand at room temperature. From time to time samples were precipitated with acetic acid and ether and the liberated phenylthiohydantoin could be estimated spectrophotometrically in the supernatant[3]. The amino acid was identified by

paper chromatography of its phenylthiohydantoin and of the acid itself after hydrolysis with $Ba(OH)_2$ or HBr. This cycle of operations was then repeated in the case of myoglobin globin. The results are summarised in Table I.

TABLE I

AMINO ACIDS LIBERATED DURING EDMAN DEGRADATION OF HORSE MYOGLOBIN AND HAEMOGLOBIN

	Myoglobin globin	
Cycle 1	Glycine – 0.6 moles	
Cycle 2	Leucine – 0.8 moles	
Cycle 3	Serine, alanine – 0.3 moles Trace of leucine.	
Cycle 4	Nil	
Cycles 5–7	...*	
	Haemoglobin	*Haemoglobin globin*
Cycle 1	Valine – 6.5 moles Valine – 6.3 moles**	Valine with trace of glycine

* In another experiment, small quantities of serine and alanine continue to appear.

** Phenylthiohydantoin of valine split off in guanidine-HCl: 1 *N* HCl[9] and continuously extracted with ether.

Results – Horse myoglobin

In horse myoglobin only glycine was found in the N-terminal position in agreement with the work of PORTER AND SANGER[4]. From the table it can be seen that the method works fairly well for the first two amino acids. Thereafter both the reaction with phenyl*iso*thiocyanate and the subsequent cyclisation give rapidly decreasing yields and in fact the reaction appears to stop after the second cycle. Small amounts of serine and alanine, its degradation product, continue to appear in subsequent stages, but these may have arisen from serine residues in the chain whose amino groups have become unmasked during previous acid treatment. ELLIOTT[8] has shown that the amino groups of serine and threonine are liberated in strongly acid solutions from silk fibroin and lysozyme, the acylating peptide fragment migrating from the α-amino group to the hydroxyl. Such amino groups would yield serine phenylthiohydantoin or the corresponding threonine derivative under the conditions of the present experiments. We may then conclude that the EDMAN degradation has not liberated the third amino acid either because the phenyl*iso*thiocyanate did not react with the amino group of the third amino acid or because the phenylthiocarbamyl derivative though formed, failed to cyclise to the thiohydantoin. It is not yet known whether all amino acids can be liberated from their peptides by the EDMAN procedure. It is also possible that the α-amino group of the third unit was formylated in anhydrous formic acid and thus blocked; it would then be necessary to assume that 90% acetic: 0.1 *N* HCl, which was used instead in another experiment, has a similar effect and that does not seem so likely.

The only definite conclusion which can be drawn from these results is that Gly-Leu- begins the N-terminal sequence of amino acids in horse myoglobin. After that the degradation method, as applied here, fails to liberate further amino acids, but the reason for this behaviour is not clear.

Results – Horse haemoglobin

It can be seen from Table I that slightly more than six valines per molecule, the expected number, were obtained as N-terminal amino acids in agreement with previous work[4]. Valine is the only amino acid in this position; the trace of glycine found in one experiment is probably not significant.

V. M. INGRAM

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge (England)

1 P. EDMAN, *Acta Chem. Scand.*, 4 (1950) 283.
2 H. N. CHRISTENSEN, *Compt. rend. trav. lab. Carlsberg*, 28 (1953) 265.
3 H. FRAENKEL-CONRAT, *J. Am. Chem. Soc.*, 76 (1954) 3606.
4 R. R. PORTER AND F. SANGER, *Biochem. J.*, 42 (1948) 287.
5 K. SCHMID, *Helv. Chim. Acta*, 32 (1949) 1198.
6 V. M. INGRAM, *Biochem. J.*, (1955) (in the press).
7 M. L. ANSON AND A. E. MIRSKY, *J. Gen. Physiol.*, 13 (1930) 469.
8 D. F. ELLIOTT, *Biochem. J.*, 50 (1952) 542.
9 H. AND J. FRAENKEL-CONRAT, *Acta Chem. Scand.*, 5 (1951) 1409.

Received February 9th, 1955