

## Human Pituitary Growth Hormone. XII. The Amino Acid Sequence of the Hormone

Sir:

In 1956, the isolation and partial characterization of human growth hormone (HGH) was described.<sup>1</sup> From sedimentation equilibrium studies<sup>2</sup> the molecular weight of the hormone was shown to be 21,500. It was also found that the hormone consists of a single polypeptide chain with four half-cystine residues.<sup>3</sup> The NH<sub>2</sub>-terminal octapeptide has been reported to be H-Phe-Pro-Thr-Ile-Pro-Leu-Ser-Arg.<sup>4</sup> By means of enzyme digestion with carboxypeptidase, the COOH-terminal residue was shown to be phenylalanine.<sup>5</sup> We wish to present herein the amino acid sequence of the HGH molecule.

pepsin. These enzymatic reactions were carried out under the same conditions as previously described.<sup>9</sup> These digests were fractionated on ion-exchange resin columns using procedures similar to those described by Schroeder, *et al.*<sup>10</sup> Some of these fractions required further purification by paper chromatography in a system consisting of 1-butanol-acetic acid-water (4:1:5, v/v) and by high-voltage electrophoresis<sup>11</sup> on paper in a buffer of pH 2.3.

Amino acid analyses of the purified peptide fragments were performed by the procedure of Spackman, Stein, and Moore<sup>12</sup> with the Spinco Model MS 120 automatic amino acid analyzer. The sequence of amino acids in these purified peptides was determined by the stepwise phenyl isothiocyanate method of Edman,<sup>13</sup> with procedures similar to those described

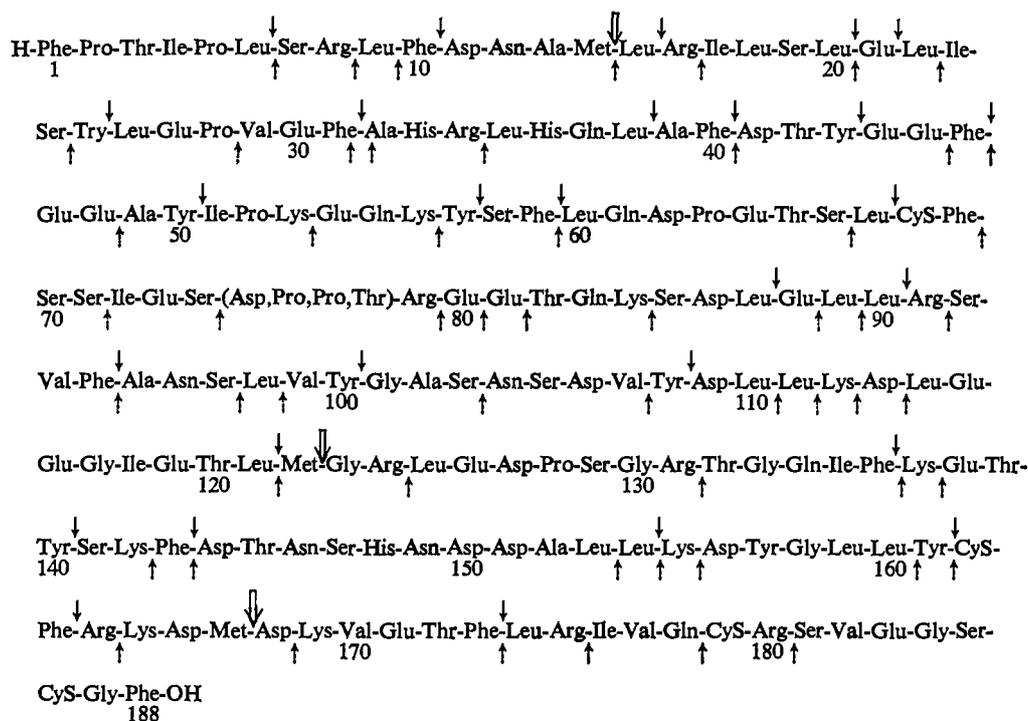


Figure 1. Amino acid sequence of human pituitary growth hormone. Numbers below the lines indicate the position of the amino acid residues from the amino terminus; ↑ indicates points of tryptic attack; ↓ indicates points of chymotryptic attack; ⇓ indicates points of peptic attack; ⌞ indicates points of attack by cyanogen bromide.

Highly purified HGH was isolated from fresh glands<sup>6</sup> by a new procedure involving gel filtration on Sephadex.<sup>7</sup> The hormone was oxidized by performic acid according to procedures already described.<sup>8</sup> Both the native hormone and the oxidized product were submitted to digestion with trypsin as well as with chymotrypsin, while only the native hormone was hydrolyzed with

by Konigsberg and Hill<sup>14</sup> or by Schroeder, *et al.*<sup>10</sup> Figure 1 indicates the cleavage of peptide bonds by trypsin, chymotrypsin, and pepsin.

In order to ascertain the proposed structure of HGH as shown in Figure 1, the hormone was allowed to react with cyanogen bromide<sup>15</sup> in 70% formic acid. Amino acid analyses indicated that the cleavage of methionyl linkage was complete. The product was submitted to oxidation by performic acid and the reaction mixture was then fractionated by gel filtration on

- (1) C. H. Li and H. Papkoff, *Science*, **124**, 1293 (1956).
- (2) C. H. Li and B. Starman, *Biochim. Biophys. Acta*, **86**, 175 (1964).
- (3) C. H. Li and W.-K. Liu, *Experientia*, **20**, 169 (1964).
- (4) W.-K. Liu, J. S. Dixon, and C. H. Li, *Biochim. Biophys. Acta*, **93**, 428 (1964).
- (5) C. H. Li, A. J. Parcells, and H. Papkoff, *J. Biol. Chem.*, **233**, 1143 (1958).
- (6) The authors wish to thank the Pituitary Bank of University of California Medical Center in San Francisco and the National Pituitary Agency in Baltimore for their generous supply of fresh human pituitary glands.
- (7) C. H. Li, W.-K. Liu, and J. S. Dixon, *Arch. Biochem. Biophys. Suppl.*, **1**, 327 (1962).
- (8) C. H. Li, *J. Biol. Chem.*, **229**, 157 (1957).

- (9) C. H. Li and G. Samuelsson, *Mol. Pharmacol.*, **1**, 47 (1965).
- (10) W. A. Schroeder, R. T. Jones, J. Cormick, and K. McCalla, *Anal. Chem.*, **43**, 1570 (1962).
- (11) A. M. Katz, W. J. Dreyer, and C. B. Anfinsen, *J. Biol. Chem.*, **234**, 2897 (1959).
- (12) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
- (13) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).
- (14) W. Konigsberg and R. J. Hill, *J. Biol. Chem.*, **234**, 2547 (1962).
- (15) E. Gross and B. Witkop, *ibid.*, **237**, 1856 (1962).

Sephadex. The amino acid composition of the purified fragments was in good agreement with that of amino acid sequences as proposed in Figure 1. Thus, the disulfide bridges are formed by residues 68–162 and 179–186. The single tryptophan residue is in position 25. The three histidine residues are in positions 33, 36, and 148. Studies on structure–activity relationship of the HGH molecule are in progress.<sup>16</sup>

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### The Structure of $(\text{NPCl}_2)_5$ . A Ten-Membered Phosphorus–Nitrogen Ring<sup>1</sup>

Sir:

The crystal structures of two members of the  $(\text{NPCl}_2)_n$  series, namely the trimer<sup>2</sup> and tetramer,<sup>3</sup> have been previously determined. While these structure determinations have been useful in partially elucidating the bonding in the phosphonitrilic chloride series, there still remain points of controversy<sup>4–6</sup> which we felt might be clarified by a structure determination of a higher member of this series. In particular, in the larger rings there are many more configurations which the molecule might assume, making the chosen configuration all the more interesting.

We have now essentially completed the single-crystal, X-ray study of  $(\text{NPCl}_2)_5$ . The crystals are orthorhombic with four molecules in a unit cell of dimensions:  $a = 15.48$ ,  $b = 19.44$ , and  $c = 6.26$  Å, and with space group symmetry  $P2_12_12_1$ . Three-dimensional data were collected using Mo  $K\alpha$  radiation with a General Electric XRD-5 X-ray unit equipped with a single-crystal orienter. The 1319 observed reflections were used to generate a sharpened Patterson. The Patterson function was deconvoluted using a symmetry map (first-order consistency function) generated from the three Harker sections<sup>7</sup> and employing superposition methods along with successive electron density map calculations. Isotropic refinement lowered the reliability factor,  $R = \sum ||F_o| - |F_c|| / \sum |F_o|$ , to 0.15 based on all observed data. Further anisotropic refinement resulted in a decrease in this factor to a final value of 0.08. Details of the structure determination and refinement will be reported later.

Decachloropentaphosphonitrile exists as a ten-membered ring consisting of alternating phosphorus and nitrogen atoms, with two chlorine atoms attached to each phosphorus (see Figure 1). The ten atoms forming the ring lie surprisingly close to their least-squares plane. Only one atom, P(5), is off the plane by more

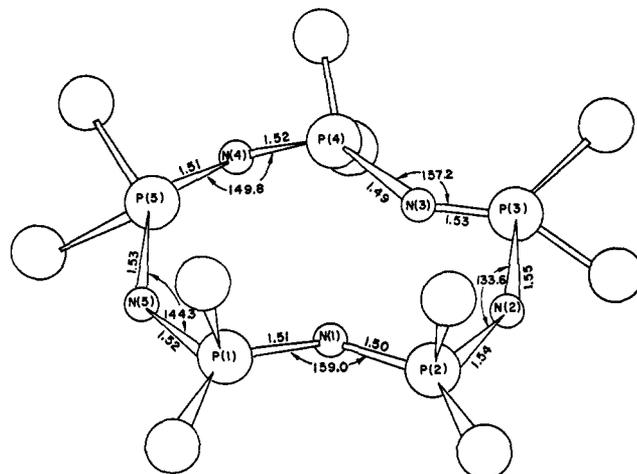


Figure 1. The molecular configuration of  $(\text{NPCl}_2)_5$ .

than 0.2 Å, and only four (P(5), P(3), N(4), and N(5)) are off by more than 0.1 Å. The five nitrogen atoms define very well a plane, the greatest deviation from this least-squares plane being less than 0.05-Å. The Cl–P–Cl and N–P–N angles are consistent with those observed in the trimer and tetramer, being 102.0° and 118.4°, respectively. The P–Cl distances found in the pentamer were slightly shorter than those in the other compounds, ranging from 1.94 to 1.98 Å. However, when thermal corrections are applied, the actual deviations will be smaller and the average distance will be closer to the 1.985 observed in the trimer and tetramer. A more serious disparity between the pentamer and the others is seen in the P–N distances and the P–N–P angles. The distances range from 1.49 to 1.55 Å, but in no systematic way as to imply alternating double-bond character. The average P–N distance of 1.52 Å is considerably shorter than the 1.59 observed in the trimer and 1.58 found in the tetramer. The average P–N–P angle in the pentamer is 148°, 16° greater than in the tetramer. Because this angle has opened up to such an extent, the pentamer is able to remain nearly planar. These observations seem to imply that the  $\pi$  character of the molecule requires a planar configuration and further that the  $\pi$ -bond character increases with increasing ring size. A more complete discussion of the structure and of the bonding as related to the structures of the lower members of the series will be reported later.

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### Steric Inhibition of the Interaction of a Cyclopropyl Substituent with the Electron-Deficient Center in the Solvolysis of *t*-Cumyl Derivatives

Sir:

There is increasing evidence that the maximum interaction between a cyclopropane group and an adjacent

(1) This work was performed in the Ames Laboratory of the U. S. Atomic Energy Commission. Contribution No. 1860.

(2) A. Wilson and D. F. Carroll, *J. Chem. Soc.*, 2548 (1960).

(3) R. Hazekamp, T. Migchelsen, and A. Vos, *Acta Cryst.*, **15**, 539 (1962).

(4) M. J. S. Dewar, E. A. C. Lucken, and M. A. W. Whitehead, *J. Chem. Soc.*, 2423 (1960).

(5) D. P. Craig and N. L. Paddock, *ibid.*, 4118 (1962).

(6) J. K. Jacques, M. F. Mole, and N. L. Paddock, *ibid.*, 2112 (1965).

(7) A. D. Mighell and R. A. Jacobson, *Acta Cryst.*, **16**, 443 (1963).