where $w^\circ(r)$ is the probability density for the harmonic case, and the a_k's are explicitly given as functions of the temperature T, the vibrational frequency ω_e for the corresponding harmonic oscillator, and the spectroscopic anharmonicity constant x_e.

If the most probable value of R is denoted by R_m, and the values of R corresponding to a probability density equal to one half of the maximum value are denoted by $R_{h,1}$ and $R_{h,2}$, it is found that to terms of second order

$$R_{h,1} - R_m = r_h^\circ(1 + x_e^{1/2}b_1 + x_e b_2),$$
$$R_m - R_{h,2} = r_h^\circ(1 - x_e^{1/2}b_1 + x_e b_2),$$

where r_h° is the half width for the harmonic case, and the b_k's are functions of the temperature T and the vibrational frequency ω_e. The probability density curve is, as it is seen, unsymmetric, the unsymmetry appearing already in a first order treatment.

Furthermore, explicit expressions have been developed for the mean amplitudes

$$u_e = [\overline{(R-R_e)^2}]^{1/2},$$
$$u_a = [\overline{(R-\bar{R})^2}]^{1/2},$$
$$u_m = [\overline{(R-R_m)^2}]^{1/2},$$

where the reference values of the interatomic distance in the three cases are the equilibrium value R_e, the mean value $\bar{R}$, and the most probable value R_m, respectively.

In case b) of the Morse-potential approximation the calculations have been carried out in a straightforward manner, considering only the lower vibrational states. However, the numerical results obtained by the two methods seem to be in very good agreement, as indicated by the values for the hydrogen molecule listed in Table 1. The necessary spectroscopic data have been obtained from Herzberg [4]. The values obtained when assuming harmonic vibrations are also given.

Table 1. Numerical data for hydrogen in Å units at $T = 300$ °K.

	Harm. osc.	Pert. meth.	Morse pot.
u_e	0.0872	0.0915	0.0917
u_a	0.0872	0.0890	0.0891
u_m	0.0872	0.0893	0.0894
$R_{h,1}-R_m$	0.1027	0.1075	0.1075
$R_m-R_{h,2}$	0.1027	0.1009	0.1008

A more detailed report on the calculations performed is to be published in *Kgl. Norske Videnskab. Selskab Skrifter.*

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N-Terminal Amino Acids of Human Pituitary Growth Hormone

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Analyses of the N-terminal amino acids of growth hormone prepared from ox pituitaries have been carried out by means of two methods. The dinitrofluorobenzene-technique (DNFB) of Sanger [1] has been applied by Li [2], who found two amino acids (phenylalanine and alanine) in equal molar proportions. These results were confirmed by the phenylthiohydantoin (PTH) method of Edman [3]. The latter method proved to be better since it was clearly demonstrable that the two amino acids were present as one mole each per mole of protein hormone [4].

This suggests a constitution formula of the ox hormone of two peptide chains linked together by SH-groups and ending in phenylalanine and alanine as N-terminal amino acids.

The following experiments were carried out to analyse the N-terminal amino acid of human pituitary growth hormone [5].

Material and methods. Growth hormone was prepared from human pituitaries by means of a method of Li and Papkoff [6]. 4 to 10 mg samples of the protein hormone were dissolved in 0.5—2.0 ml of phosphate buffer pH 9.0, ionic strength 0.1. An equal volume of 0.5 % phenylthio*iso*cyanate (Eastman Kodak) in ethanol was added and the mixture stirred for 2 h. The pH was maintained at 8.8 by the addition of small portions of 0.01 N sodium

hydroxide. A Beckman autotitrator apparatus was used for the checking of pH and addition of alkali. When the alkali consumption had ceased three volumes of ethanol were added and the pH was lowered to 4 by addition of hydrochloric acid. After centrifugation the PTC-protein was washed three times with ethanol and lyophilized.

10 ml of N hydrochloric acid was added to the dry PTC-protein powder and the mixture was refluxed on a sandbath for 2 h. After cooling the PTH-amino acids were extracted with three 10 ml portions of ethyl acetate. The combined extracts were evaporated to dryness *in vacuo*. The dry residue was dissolved in a small volume of ethyl acetate and applied for chromatography on formamide-buffered Whatman No. 1 papers and developed with descending chromatography using xylene as a solvent [7].

Standard PTH-amino acids were always run together with the unknown. Indication of the spots was made by means of ultraviolet absorption with the aid of a fluorescence screen. For quantitative estimation of the N-terminal amino acid various amounts of the PTH-amino acid were run together with the preparations. The spots were eluated with 70 % ethanol, the volume made up to 10 ml and samples thereof read against ethanol in a Beckman spectrophotometer model DU at 269 mμ. Care was taken to make the paper pieces of exactly the same area since extracts from the formamide-buffered paper has a high blank absorption. The area of filter paper used usually had an absorption value of 0.2 (See Fig. 1).

Parts of the PTH-amino acid extracts were boiled in 2 N sodium hydroxide for 3 h in order to transform them into the original amino acid. Identification of the amino acid was made by two-dimensional paper chromatography using Whatman No. 4 paper, water-saturated phenol and collidine-lutidine according to Dent [8]. The standard PTH-amino acids used were synthetisized according to Edman [3] and the purity was checked with melting point determination and paper chromatography.

Results. Only one N-terminal amino acid was demonstrable as a PTH-preparation by the method used. The identification of this amino acid proved to be rather troublesome. Use of xylene as a solvent in the paper chromatography showed a spot with a R_F-value in the vicinity of PTH-valine or PTH-phenylalanine. Other solvents (formic acid, *n*-butanol and hexane in various proportions according to Sjöquist [9]) did not give a reliable identification though the results of chromatography

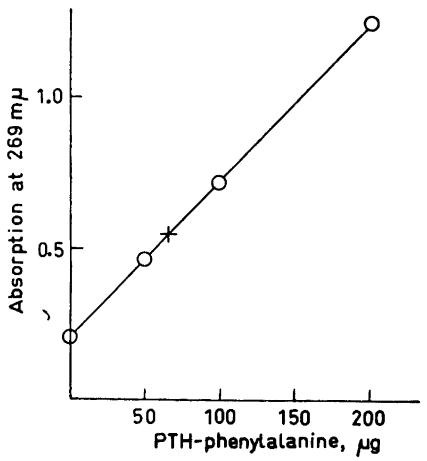

Fig. 1. Absorption at 269 mμ of a PTH-preparation from 10.0 mg of growth hormone and a standard PTH-phenylalanine preparation.

The PTH-phenylalanine from 10.0 mg of growth hormone had an absorption value of 0.550 which corresponds to 65 μg of PTH-phenylalanine (+) or 35.9 μg of phenylalanine. Thus one protein molecule contains 10 000/35.9 = 278 amino acids which means a molecular weight of about 30 600.

with xylene were confirmed. Breaking down the PTH-amino acid to the original amino acid by alkali and chromatography according to Dent [8] clearly showed the presence of phenylalanine. Quantitative determination of the PTH-phenylalanine showed that one mole of phenylalanine was present as N-terminal amino acid per mole of protein hormone.

Discussion. This study shows the presence of phenylalanine as N-terminal amino acid of human pituitary growth hormone. In contrast to the growth hormone of ox pituitaries this indicates the possibility that only one N-terminal amino acid per mole of protein is present. Thus the structure of the human growth hormone seems to differ from that of ox hormone. As already has been pointed out the ox hormone consists of two peptide chains. The human hormone, however, seems to be built up as a single peptide chain ending in phenylalanine as N-terminal amino acid. By means of the DNFB-technique phenylalanine has been demon-

strated in the human growth hormone [6]. The molecular weight calculated on basis of the quantitative analysis of N-terminal amino acid gave a value of 30 600 which is in accordance with previously reported values [10] (See Fig. 1).

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On the Crystal Structure of $POCl_3 \cdot SbCl_5$

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Single crystals of $POCl_3 \cdot SbCl_5$ were prepared in capillary tubes by a method of zone melting. Rotation and Weissenberg photographs were taken around the *c*-axes with Mo-*K* and Cu-*K* radiation. The orthorhombic unit cell has the dimensions $a = 8.06 \pm 0.01$ Å, $b = 16.42 \pm 0.01$ Å and $c = 8.93 \pm 0.02$ Å. These values are in agreement with the preliminary values found earlier [1] ($a = \frac{1}{2} = 8.1$ Å, $b = 16.2$ Å and $c = 8.8$ Å). The extinctions $h0l$ for $h + l$ odd, and $hk0$ for k odd are in agreement with the centrosymmetric space-group $P\,m\,n\,b$ which has been confirmed by the structure determination. (This space group was by mistake not found in the earlier investigation [1]).

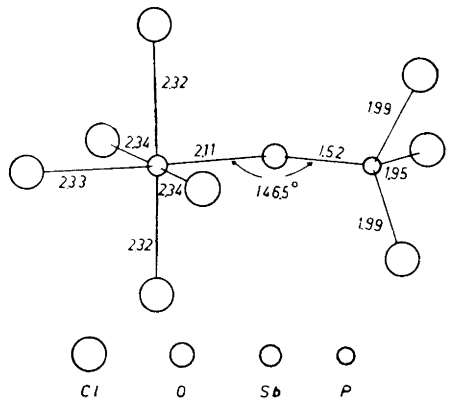

Fig. 1.

The number of molecules in the unit cell has been shown [1] to be 4.

The structure was determined from three-dimensional Patterson functions and confirmed by a three-dimensional electron density calculation. The unrefined parameters are:

4 Sb	in	$x = 0.250$	$y = 0.145$	$z = 0.079$
4 Cl	in	$x = 0.250$	$y = 0.257$	$z = 0.919$
4 Cl	in	$x = 0.250$	$y = 0.021$	$z = 0.207$
4 Cl	in	$x = 0.250$	$y = 0.226$	$z = 0.294$
8 Cl	in	$x = 0.538$	$y = 0.143$	$z = 0.070$
4 O	in	$x = 0.250$	$y = 0.073$	$z = 0.884$
4 P	in	$x = 0.250$	$y = 0.073$	$z = 0.714$
4 Cl	in	$x = 0.250$	$y = 0.464$	$z = 0.871$
8 Cl	in	$x = 0.054$	$y = 0.133$	$z = 0.633$

The shape of the $POCl_3 \cdot SbCl_5$ molecule and the interatomic distances within the molecule are given in Fig. 1. The structure is built up by the packing of such molecules. The complete structure determination will be published after refinement of the parameters.

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