forming a part of the d^5-hump energy differences, actually decrease in anion complexes, compared to complexes of neutral ligands [10], it cannot be excluded that this effect will produce a slight decrease of ligand field stabilization in the complexes with stronger partial covalent bonding.

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Table 1.

PTH-amino acid	R_F-value in solvent		
	D	*E*	*F*
Glycine	0.09	0.63	0.62
Alanine	0.18	0.77	0.78
Valine	0.53	0.87	0.89
Isoleucine	0.65	0.92	0.91
Leucine	0.67	0.92	0.92
Serine	0	0.47	0.14
Threonine	0	0.55	0.34
Proline	0.83	0.90	0.89
Hydroxyproline	—	—	0.54
Methionine	0.45	0.89	0.88
Cysteic acid *	0	0	0
Phenylalanine	0.57	0.90	0.91
Tyrosine	0	0.75	0.41
Tryptophan	0.12	0.86	0.82
Aspartic acid	0	0.38	0.16
Glutamic acid	0	0.56	0.27
Asparagine	0	0.31	0.08
Glutamine	0	0.41	0.15
Histidine	0	0.22	0
Arginine	0	0.11	0
ε-PTC-lysine	0.05	0.85	0.81

* PTH-cysteic acid may be identified by paper electrophoresis.

Identification and Semiquantitative Determination of Phenyl Thiohydantoins

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In an earlier paper [1], one of us described a paper chromatographic procedure for identification of the phenyl thiohydantoin (PTH) derivatives of natural amino acids. The procedure has now been improved in a way, that permits a semiquantitative determination based on the strong ultraviolet absorption of the PTH. This called for a method of locating the PTH's on the paper without destroying them and also for a low background absorption. For the first purpose a low pressure mercury lamp and a fluorescent screen with excitation in the appropriate ultraviolet region and emission in the visible were used *. In order to cut out the visible light from the mercury lamp a dye emitting in the visible red was used and observations were made through red glasses. The PTH's then appear on the paper as black spots on a red background. The lower limit of sensitivity is approx. 1 μg of PTH in a spot**. For a semiquantitative determination at least 5 μg in each spot is required. After location of the spot the area is cut out, the material extracted from the paper and its absorption measured. The yield varies between 75 and 105 %, the lower figure usually being obtained for the PTH's with highest R_F-values.

The solvents were chosen so that after drying the paper they did not leave material absorbing in the critical UV region around 270 mμ.

* Related procedures have been described for the location of steroids [2] and nucleic acid material [3] on paper chromatograms.

** For identification purposes the iodine-azide reaction [1] should afterwards be applied.

Another possible use of this procedure, in addition to the determination of the N-terminal amino acids, may be briefly referred to here. In conjuction with a micro procedure for the synthesis of PTH's from amino acids at present being developed in our laboratory [4] it can be used for amino acid analysis. It offers the advantages of rapidity, small material requirements (0.1 —0.2 mg of protein hydrolyzate) and noninterference of salts.

Experimental. *o*-Xylene is shaken three times with 1/10 vol. of conc. H_2SO_4 for 1/2 h with cooling to below 30° C. The organic phase is then washed with water, dried over KOH pellets overnight and distilled.

n-*Butyl acetate* is distilled and the fraction boiling at 126—127° C used.

Propionic acid is refluxed for 4 h with chromic acid (10 g/750 ml), distilled on a Widmer column and the fraction boiling at 140—141° C collected.

n-*Heptane* is shaken three times with 1/10 vol. of conc. H_2SO_4 and then washed successively with water, 10 % aqueous NaOH and water. It is finally dried over Na_2SO_4 and distilled.

Ethylene chloride, puriss (Fluka).

Formic acid, analytical reagent (Merck).

Formamide, analytical reagent (Fluka).

Filter paper. Whatman No. 1. Impregnation with starch prior to use [1] does not interfere with the quantitative determinations.

Fluorescent screen. A fine suspension of fluorescent cadmium borate * in acetone-amyl acetate (2:1) is sprayed evenly on to a 500 × 500 × 2 mm celluloid sheet, dried, and the operation repeated a number of times. When a good and even coverage has been obtained a hot solution of polyethylene in heptane is sprayed on to form a protective coating. The screen is more convenient to use if put in a frame in a slightly curved position.

Mercury lamp. Original Hanau NK 20/40 with main UV emission at 254 mμ.

PTH-references. The synthesis of the PTH-amino acids has been described earlier [5-7]. A molar extinction coefficient in ethanol at 269 mμ of 14 000—16 000 applies to most PTH-amino acids with the exception of PTH-ε-phenyl thiocarbamyl lysine (ε_{269} = 29 000) and PTH-tryptophane (ε_{269} = 19 700). The reference solutions are made up in ethylene chloride (0.5—1.5 mg/ml) except PTH-arginine and PTH-histidine which owing to their low solubility in ethylene chloride are dissolved in hexanol. In solution the PTH-amino acids decompose slowly and should therefore be stored in the cold.

Solvent systems:

D. *o-Xylene/formamide.* The paper is dipped into a solution of formamide in acetone (2:7) and immediately placed horizontally on a filter paper to remove excess solution. The strip is then suspended for a few minutes in the air for the acetone to evaporate. Reference compounds and unknowns in appropriate solvents (*cf.* above) are applied to the paper, and descending elution of the chromatogram immediately started using *o*-xylene.

E. n-*Butyl acetate/propionic acid/formamide.* The paper strip is impregnated with formamide as described for solvent system *D*. The butyl acetate is saturated with water, 3 % (v/v) propionic acid added and this mixture subsequently saturated with formamide. The filter paper lining of the chromatography tank is moistened with the solvent mixture. The paper strip is allowed to equilibrate in the chamber for 1/2 h before starting development.

F. n-*Heptane/ethylene chloride/formic acid.* The upper phase obtained after mixing 1 vol. of *n*-heptane, 2 vols. of 75 % aqueous formic acid and 2 vols. of ethylene chloride is used for developing the chromatogram and the lower phase is poured on the filter paper lining of the chromatography tank. An equilibration time of 1/2 h is required.

For the semiquantitative determination a sample of the unknown (5—20 μg) is applied together with varying, known amounts of the corresponding PTH-reference compound. An empty lane serves as a blank. After development the paper is dried at 90° C for 10 min, the spots marked out on the paper with the aid of the fluorescent screen, and cut out with a margin of *ca.* 3 mm. An area corresponding in size and level is cut out from the blank lane. The spots are eluted with 1.5 ml of ethanol for 2 h at room temperature and the extinctions of the ethanolic extracts read at 269 mμ in a Beckman spectrophotometer model DU provided with micro cuvettes. The accuracy of a single determination is ± 10 % although the yield in absolute terms may be reduced to 75 % for the faster moving spots.

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* Obtainable from AB Lumalampan, Stockholm 20.

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Isotope Effect in the Hydrolysis of Grignard Compounds. II *

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Measurements of the hydrogen isotope effect in the hydrolysis of methylmagnesium iodide and phenylmagnesium iodide in a solution of anisole and pyridine were reported in a previous preliminary paper [1]. Pyridine, with its fairly basic character, and its ability to form addition products, may influence the reaction mechanism. The reactions have now been carried out in a more inert solvent, *i.e.* a mixture of anisole and tetrahydrofuran. The hydrolytic agent was in some of these experiments tritiated water and in others a tritiated mixture of water and hydrochloric acid.

The water was present in at least tenfold excess in all experiments and in the acid experiments the hydrogen chloride was present in twice the amount stoichiometrically needed. (The acid concentration in the acid-water mixture was about 18 % HCl by weight.) The technique was analogous to the one used earlier, *i.e.* the Grignard compound, dissolved in anisole-tetrahydrofuran, was added by means of an injection syringe or a dropping funnel to the water or the dilute hydrochloric acid, both dissolved in tetrahydrofuran. The anisole had been dried according to current methods, while the drying of the tetrahydrofuran was carried out by shaking it with anhydrous $CaCl_2$, thereafter with metallic sodium and finally by distilling it from methylmagnesium iodide. After the preparation of the phenylmagnesium halide, a fraction of the solvent was distilled off at reduced pressure in order to eliminate benzene formed by moisture or other side reactions. The mixture of anisole and tetrahydrofuran in the proportion used

* Ref. [1] is considered as part I.

Table 1. Results in the decomposition of Grignard compounds with water or hydrochloric acid at 20°C.

Grignard compound	Hydrolysis by	k_T/k_H	Number of parallel determinations of tritium in combustion water	Mean value (including error in determination of tritium in initial water)
CH_3MgI	water	0.700	2	0.71 ± 0.02
		0.713	2	
	hydrochloric acid	0.710	1	0.71 ± 0.04
		0.694	1	
		0.738	1	
C_6H_5MgI	water	0.644	1	(0.64 ± 0.04)*
C_6H_5MgBr	water	0.561	2	0.57 ± 0.02
		0.570	1	
	hydrochloric acid	0.579	2	0.57 ± 0.02
		0.567	1	

* Only one experiment.