peptides. During an automated degradation the sequencer can deliver several PTH amino acids in 24 h, which must be identified rapidly to match the output. In view of the limited space in this review, the method of formation of a PTH derivative from an amino acid and from the N-terminal end of a polypeptide is only briefly discussed in the following subsection. It follows the results of some successful TLC systems used for resolution and identification of PTH amino acids. The PTH amino acids are sensitive to light, and optically active derivatives racemize easily.

## A. PTH Amino Acids

### 1. Preparation of PTH Amino Acids*

Amino acid (0.5–1.0 g) is added to aqueous pyridine (1:1) (25 mL) in a stoppered tube. The solution is adjusted to pH 9.0 with 1 N NaOH and placed in a water bath at 40°C. Phenyl isothiocyanate (1.2 mL) is added with shaking during a reaction time of about 30 min. Additional alkali is added to maintain the pH at 9. The mixture is extracted repeatedly with benzene to remove excess reagent and pyridine. When there is no further uptake of alkali, a slight excess of 1 N HCl is added to precipitate the PTC amino acid. The mixture is filtered and warmed with HCl (1 N, 30 mL) at 40°C for 2 h. The PTH derivative crystallizes upon cooling, and further yields are obtained by concentration of mother liquors. Most of the derivatives are recrystallized from aqueous acetic acid or ethanol.

The PTH derivatives of serine, threonine, and cystine are extremely labile. Ingram (92) applied milder conditions for serine and threonine. These were condensed with phenyl isothiocyanate at room temperature, and then the pH was brought to 1. Some pink oil was separated and discarded. The reaction was allowed to proceed for 2 days at room temperature, when PTH derivatives crystallized out. Sjoquist (78) described a method for microlevel preparation of PTH amino acids.

### 2. PTH Amino Acids from N-Terminal Polypeptides

Since the original report of Edman (76), many modifications to the experimental conditions have been reported (93–95). The technique developed by Fraenkel-Conrat and Harris (93) has been used successfully in this laboratory (96,97) and is described below.

The peptide (0.2–0.3 mg) is dissolved in aqueous dioxane (50%, 4 mL), and the pH is adjusted to 8.7–9.0 with 0.01 N NaOH. The mixture is stirred for 1.5 h at 40°C with phenyl isothiocyanate (0.1 mL), keeping the pH constant. The reaction mixture is extracted seven times with benzene, and the aqueous solution is concentrated to dryness in vacuo.

The sodium salt of PTC peptide is redissolved in water (2–10 mL), and aliquots corresponding to 0.2–1.0 $\mu$M are made 3 N with respect to hydrochloric acid and 0.2–1.0 $\times$ $10^{-4}$ M with respect to peptide by addition of the correct amounts of water and 5.7 N HCl. The rate of release of phenylthiohydantoin can be determined by following the change of the absorption maximum of the solution (from 240 nm or lower to 265–270 nm) over a period of about 2 h. If the transformation takes place too slowly for a given peptide, the effect of increasing the temperature to 40–45°C should be investigated.

The PTH amino acids are extracted with ethyl acetate (with the exception of PTH arginine and PTH histidine), and residual peptide is recovered by concentration of the aqueous solution. The residue is redissolved in 50% aqueous dioxane and submitted to the same cycle of operations.

### 3. TLC Resolution, Detection, and Identification of PTH Amino Acids

Thin-layer chromatography has been used for the identification of PTH amino acids since Edman and Begg (98) used it in their classical work describing the automatic sequencer. TLC of PTH amino acids has been reviewed by Rosmus and Deyl (99), Niederwieser (100), Allen (101), and Bhushan and Reddy (102). Various TLC systems with different kinds of adsorbents, such as alumina, silica gel, and polyamide, have been reported. The methods of detection include (a) spraying a dilute solution of fluorescein on a plain layer of silica gel so the spots become visible

*After Ref. 76.