**Table 8** Characteristic Colors of PTH Amino Acids Following Ninhydrin Spray

| PTH derivative | Color properties | $NH_4OH$ color change |
|---|---|---|
| Proline | UV, colorless | Light blue after heating |
| Alanine | Purple | Deeper color |
| Glycine | Orange | |
| Serine | UV, purple | |
| Serine breakdown | Faint orange | Weak red |
| Asparagine | Yellow | More intense |
| Carboxymethylcysteine | UV, purple | |
| Methioninesulfone | Light tan | |
| Methionine | Faint tan | |
| Lysine | Very faint pink | Weak blue after heating |
| Tyrosine | UV, yellow before spray | Intense yellow |
| Threonine | Colorless | Light tan |
| Glutamine | Dark green | Dark blue |
| Phenylalanine | UV, colorless | Faint yellow |
| Tryptophan | UV, yellow before spray | Deep yellow |
| Aspartic acid | UV, pink | Darker |
| Glutamic acid | Gray | Dark blue |

Silica gel plates, without fluorescent indicator, developed in heptane–$CH_2Cl_2$–propionic acid (45:25:30) and xylene–MeOH (80:10), sprayed with iodine azide and 1.7% ninhydrin in MeOH–collidine–HOAc (15:2:5), heated at 90°C for 20 min; color changes induced by blowing a saturated ammonia atmosphere over the ninhydrin plate.
*Source*: Ref. 104.

Resolution and identification of PTH amino acids on silica or polyamide layers by TLC as discussed above showed difficulties in achieving discrimination between derivatives of Leu/Ile (106) and resolution of complex mixtures without two-dimensional chromatography (113). Also, difficulties in resolving combinations of PTH-Phe/Val/Met/Thr (114,115) and PTH-Asp and -Glu were observed. The use of chloroform–acetic acid (27:3) and chloroform–methanol (30:4) has been found extremely satisfactory for the discrimination between PTH-Asp and PTH-Glu, because the difference in their $hR_f$ values was around 10 units (116). The difficulties, previously posed and as noted above, in resolving and identifying various combinations of PTH amino acids can be overcome by the use of certain solvent systems (30a,30b) given in Table 9.

## B. Dansyl Amino Acids

Derivatization of free amino group of amino acids with 5-methylamino naphthalene-1-sulfonyl (dansyl) chloride has become increasingly popular for N-terminal end determinations in proteins and for manual Edman degradation (91). The dansylation reaction has also been used as one of the most sensitive methods for quantitative amino acid analysis (117,118).

1. Dansylation of Peptides*

The peptide is dissolved in a small volume of 1% (v/v) aqueous triethylamine, and a small aliquot (1 μL, 0.5 nmol) is transferred to a dansyl tube (4 × 50 mm) that has been preheated at 500–600°C overnight. The sample is dried, and sodium bicarbonate (0.2 M, 3 μL) and dansyl chloride (3 μL) solution (5 mg/mL in dry acetone) are added. The tube is sealed with parafilm and incu-

*Method of dansylation from Ref. 119.