Detection of tert.-butyloxycarbonyl derivatives on paper and thin-layer chromatograms

Since the *tert.*-butyloxycarbonyl (*t*-Boc) group was first introduced as an amino nitrogen protecting group in peptide synthesis it has gained very wide usage and has become second only to the benzyloxycarbonyl group[1]. Recently it has been shown that *tert.*-alkyloxycarbonyl derivatives of amino acids and peptides, besides being very sensitive towards acid, are also heat sensitive. The *tert.*-alkyloxycarbonyl group can be removed thermally either in the fused state or in aqueous solution to give the corresponding free amino acid or peptide in over 90 % yield[2].

Under the usual conditions, both paper and thin-layer chromatograms of *tert.*-butyloxycarbonyl derivatives of amino acid and peptides give a negative ninhydrin test[3]. We have found that a positive ninhydrin test is obtained if the paper or the thin-layer plate was heated at 125–130° for some time, before or after spraying with the ninhydrin reagent.

TABLE I

R_F VALUES OF *tert.*-BUTYLOXYCARBONYL DERIVATIVES ON PAPER CHROMATOGRAMS

Derivative	*Solvent A (butanol–acetic acid–water)*[4]	*Solvent B (sec.-butanol–ammonia*[5]*)*
t-Boc·Gly·OH	0.90	0.53
t-Boc·Leu·OH	0.95	0.74
t-Boc·Try·OH	0.93	0.66
t-Boc·Tyr·OBz	0.85	0.95
t-Boc Lys·Ala	0.82	0.78
t-Boc·Lys(Z)·Lys(Z)·Ala·OMe	0.89	0.91
t-Boc·Leu·Lys(Z)·Lys(Z)·Ala·OMe	0.92	0.92

In the case of paper chromatograms the best results were obtained by first spraying the dry paper with the ninhydrin solution and then heating it in an oven at 125–130° for 25 min. R_F's of several *tert.*-butyloxycarbonyl derivatives, which have been obtained using Whatman No. 1 filter paper, are given in Table I.

After heating thin-layer chromatograms of *tert.*-butyloxycarbonyl derivatives at 125–130° for 25 min, positive ninhydrin spots are obtained upon spraying the hot plates with an 0.25 % solution of ninhydrin in butanol. Some of the R_F's which have been obtained using 20 × 20 cm glass plates coated with an 0.25 mm layer of silica gel G (E. Merck and Co., Darmstadt, Germany) are given in Table II.

TABLE II

R_F VALUES OF *tert.*-BUTYLOXYCARBONYL DERIVATIVES ON THIN-LAYER CHROMATOGRAMS

Derivative	*Solvent A (methanol–water (9:1))*	*Solvent B (dioxan–water (6:4))*	*Solvent C (sec.-butanol–ammonia)*[5]
t-Boc·Gly·OH	0.62	0.83	0.37
t-Boc·Leu·OH	0.61	0.74	0.49
t-Boc·iLeu·OH	0.62	0.83	0.49
t-Boc·Lys(Z)·OH	0.57	0.81	0.52
Z·Lys(*t*-Boc)·OH	0.62	0.87	0.77
t-Boc·Thr·O⁻DCHAH⁺	0.65	0.84	0.40
t-Boc·Try·OH	0.70	0.84	0.45
t-Boc-Lys·Ala-	0.63	0.81	0.61
Z·Lys(*t*-Boc)·His·OMe	0.75	0.84	0.73
t-Boc·Lys(Z)·Lys(Z)·Ala·OMe	0.82	0.92	0.77
t-Boc·Leu·Lys(Z)·Lys(Z)·Ala·OMe	0.83	0.89	0.85

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