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Note

Dicarboxidine [γ,γ'-(4,4'-diamino-3,3'-biphenylylenedioxy)dibutyric acid] dihydrochloride as a chromogen for the detection of N-tert.-butyloxycarbonyl amino acids and peptides on thin-layer chromatograms

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The chlorine–*o*-tolidine method[1,2] for the detection on thin-layer chromatograms of compounds that contain groups such as –CONH– which can be transformed into chloramines is frequently applied to N-protected amino acids and peptides. The carcinogenic properties of *o*-tolidine require, however, that this compound be replaced with safer alternatives. Such a compound, γ,γ'-(4,4'-diamino-3,3'-biphenylylenedioxydibutyric acid dihydrochloride (dicarboxidine), has recently been described[3]. We have now compared this compound with *o*-tolidine as chromogen for the detection of N-*tert.*-butyloxycarbonyl(BOC)-protected amino acids and peptides.

EXPERIMENTAL

The N-BOC-amino acids selected were derivatives of simple aliphatic amino acids lacking groups that could be easily detected by other techniques. N-BOC-L-alanine (1), N-BOC-L-glycine (2), N-BOC-L-isoleucine (3), N-BOC-L-proline (4), N-BOC-S-acetamidomethyl-L-cysteine (5), N-BOC-L-glutaminic acid γ-benzyl ester (6), N-BOC-L-glutamic acid γ-*tert.*-butyl ester (7) and pyroglutamic acid (8), were commercial products. *p*-Glu–Ser–Gly–NH_2 (9), H–Leu–Gly–Gly–Ala–Lys–Gln–Ala–Gly–Asp–Val–OH (10) and the tetradecapeptide somatostatine (H–Ala–Gly–Cys–Lys–Asn–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser–Cys–OH) (11) were obtained from G. Westin-Sjödahl and R. Lundin (Recip Polypeptide Laboratory, AB Kabi, Stockholm, Sweden).

N-BOC-amino acids were dissolved in chloroform and diluted so that by applying 1 or 2 μl to the chromatograms the amounts obtained in the spots were 20, 10, 1, 0.5, 0.2 and 0.1 μg. Compound 8 and the peptides were dissolved in 0.2 *N* acetic acid and diluted so that by applying 1 or 5 μl the amounts obtained in the spots were 5, 1, 0.5 and 0.1 μg.

The eluents used with silica gel 60 F pre-coated TLC plates (Merck, Darmstadt, G.F.R.) were chloroform–acetic acid (9:1) for the N-BOC-amino acids and ethyl acetate–pyridine–water–acetic acid (5:5:3:1) for 8 and the peptides. The *o*-tolidine spray reagent was prepared according to Krebs *et al.*[4], and the dicarboxidine spray reagent was prepared by dissolving 500 mg of dicarboxidine in a mixture of 30 ml of water and 20 ml of acetic acid containing 1 g of potassium iodide.

The chromatograms were developed for a distance of 13 cm and air dried thoroughly (2 h for the pyridine-containing solvent) before the chlorination, which was performed as described by Krebs *et al.*[4].

RESULTS

The R_F values and detectable amounts of N-BOC-amino acids and peptides are given in Table I.

TABLE I

R_F VALUES AND DETECTABLE AMOUNTS

Compound No.	R_F	*Amount (μg) applied**						
		20	*10*	*5*	*1*	*0.5*	*0.2*	*0.1*
1	0.47	+, ×	+, ×		+, ×	+, ×	+, ×	+, ×
2	0.29	+, ×	+, ×		+, ×	+, ×	+, ×	+, ×
3	0.66	+, ×	+, ×		+, ×	(+), ×	−, (×)	−, −
4		−, −	−, −	−, −	−, −	−, −	−, −	−, −
5	0.05	+, ×	+, ×		+, ×	+, ×	+, ×	+, ×
6	0.56	+, ×	+, ×		+, ×	+, ×	+, ×	−, ×
7	0.48	+, ×	+, ×		+, ×	+, ×	+, ×	−, ×
8	0.36			+, ×	+, ×	+, ×		−, ×
9	0.43			+, ×	+, ×	+, ×		(+), (×)
10	0.54			+, ×	+, ×	+, ×		(+), (×)
11	0.20			+, ×	+, ×	+, ×		(+), (×)

* + = Visible and (+) = barely visible with *o*-tolidine; × = visible and (×) = barely visible with dicarboxidine; − = not visible.

With *o*-tolidine, all of the tested compounds appeared as violet spots, sometimes with a yellow core at high concentrations (for 1 at 20 μg, for 2 at 10 μg, for 6 at 20 μg, for the peptides at 5 μg and for somatostatine at 1 μg). With dicarboxidine, the N-BOC-amino acids appeared as brownish violet spots with a red-brown core at high concentrations (for 1 at 20 μg and 2 at 10 μg). The peptides appeared as greenish violet spots with a brown core at high concentrations (5 μg). Compound 4 was invisible at all concentrations, as expected.

CONCLUSION

The results indicate that the sensitivity of the chlorine–dicarboxidine spray reagent is as high as that of the chlorine–*o*-tolidine spray reagent. Dicarboxidine can therefore be recommended as a safer substitute for the carcinogenic *o*-tolidine.

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