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## The detection of benzyloxycarbonyl-protected amino acid and peptide derivatives on thin-layer chromatograms

The application of thin-layer chromatography to peptide chemistry has been the subject of a recent monograph[1].

The classical benzyloxycarbonyl group, introduced by BERGMANN AND ZERVAS[2], is still the most common amino-protecting group used in the synthesis of peptides. We have for some time been investigating the possibility of detecting benzyloxycarbonyl-protected amino acid and peptide derivatives on chromatographic plates using ninhydrin. Our original plan was either to add a deblocking agent to the ninhydrin solution or to follow spraying with a deblocking reagent by treatment with ninhydrin. After inspection of the methods already developed for the removal of benzyloxycarbonyl groups in preparative work, it seemed possible to us that an acid, such as trifluoroacetic acid (WEYGAND AND STEGLICH[3]), would be worth testing. We chose for this purpose the less volatile trichloroacetic acid. In this way, by either using a ninhydrin solution in *n*-butanol containing 10% trichloroacetic acid, followed by heating to 100°, or by spraying with a 10% solution of trichloroacetic acid in glacial acetic acid, followed by heating to 100° and then spraying with ninhydrin, satisfactory spots could be obtained from some benzyloxycarbonyl compounds. However, a cleaner procedure, not involving the unpleasant trichloroacetic acid, seemed desirable.

Very recently WOLMAN AND KLAUSNER[4] published a procedure for the detection of *tert.*-butyloxycarbonyl derivatives on chromatograms, based on the sensitivity of these compounds to heat. Thus, after heating thin-layer chromatograms to 125–130° for 25 min, ninhydrin-positive spots were obtained.

We have now found that benzyloxycarbonyl compounds, too, are sensitive to heat. A somewhat higher temperature is needed for most benzyloxycarbonyl compounds than for *tert.*-butyloxycarbonyl compounds, although some can be detected

under the conditions used by WOLMAN AND KLAUSNER, especially if the quantity of compound present on the plate is not too small.

To determine the temperature conditions needed to decompose benzyloxycarbonyl compounds, 1, 10 and 100 $\mu$g of benzyloxycarbonyl-L-aspartic acid, benzyloxycarbonyl-L-proline, benzyloxycarbonyl-L-serine and benzyloxycarbonylglycine (also 0.1 $\mu$g of the last named), dissolved in 1 $\mu$l of acetone, were spotted on plates, coated with Kieselgel G (Merck), and run in the system abs. ethanol–water (7:3), used by PATAKI[5]. The plates were heated to different temperatures for 30 min, sprayed with a 0.25 % solution of ninhydrin in *n*-butanol, and dried for further 15 min at 100°. Of the thirteen solutions applied to each plate, three gave spots on a plate heated to 100°, nine on a plate heated to 125°, while all thirteen gave spots on plates heated to 150, 175 and 200°. An increase in the intensities of the spots with heating temperature was quite clear up to 175°, while the plate heated to 200° only gave better spots for the proline compound. Two more plates, heated to 175 and 200°, were sprayed with a 0.2 % solution of isatin in acetone and dried at 75° for 15 min. In this case, too, there was some difference in the intensities of the proline spots.

1 and 10 $\mu$g of the benzyloxycarbonyl derivatives of following compounds were applied to Kieselgel G plates, which were then developed to about 10 cm in the two systems *n*-butanol–acetic acid–water (4:1:1) and *n*-propanol–water (7:3): $\beta$-alanine, glycyl-glycine, L-alanyl-L-phenylalanine, L-valyl-L-leucine, glycyl-glycine N-hydroxysuccinimide ester, glycyl-glycyl-L-seryl-L-proline *tert.*-butyl ester, L-seryl-L-proline *tert.*-butyl ester, L-proline *tert.*-butyl ester and L-prolyl-L-proline methyl ester. After heating to 200° for 30 min, spraying with a 0.25 % ninhydrin solution in *n*-butanol and heating to 100° for 15 min, spots were obtained from all the compounds except the last. 1 $\mu$g of this compound could definitely not be detected and 10 $\mu$g only gave a faint spot. 1 $\mu$g of the first four compounds gave distinct spots, whereas this amount of the other seemed to be very near to the limit of detection.

Owing to the simplicity of the above procedure and the sensitivity of amino acids and most smaller peptides to ninhydrin, we believe the method described to be a complement to other less specific methods used to detect benzyloxycarbonyl compounds on thin-layer chromatograms.

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