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Note

Selective and highly sensitive spray reagent for detection of nanomolar quantities of carbodiimides on thin-layer plates

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In the course of our investigations we required several peptides, which had to be absolutely uncontaminated by the carbodiimides employed during synthesis. In order to follow the complete disappearance of carbodiimides during the reaction, a selective and highly sensitive spray reagent had to be developed, since no suitable assay for carbodiimides on thin-layer chromatographic (TLC) plates had previously been reported. Such an assay could be of general interest since, upon charring of silica plates, carbodiimides leave no traces, and remain undetected when reaction mixtures are assayed.

Recently the reaction between carbodiimides, pyridine and barbituric acid or *N,N'*-dimethylbarbituric acid was employed for the determination of carbodiimides in solution¹. We found that the same reaction, which results in the formation of an intensely coloured purple dye ($\epsilon \approx 150,000 \text{ l mol}^{-1} \text{ cm}^{-1}$), can also be used for the detection of carbodiimides on TLC plates. Screening for interfering substances showed that urea forms derivatives, like dicyclohexylurea, imidocarbonates, carbamates, isourea derivatives and α -amino acids, which are totally unreactive. Carbodiimides¹, cyanates (R-OCN)², triazine derivatives² and cyanogen halides^{3,4} seem to be the only compounds which react with the pyridine-barbituric acid reagent. Of these compounds only the carbodiimides are commonly used in peptide synthesis. On TLC silica gel sheets the reaction is highly sensitive. Using dicyclohexylcarbodiimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as models for water-insoluble and water-soluble carbodiimides respectively, the formation of clearly visible purple spots allowed the detection of as little as 1 nmol of carbodiimide ($\approx 0.2 \mu\text{g}$). These spots exhibited a strong cherry-red fluorescence, and remained visible for several hours up to several weeks. Unfortunately they are unstable for longer periods. In terms of sensitivity, the colour reaction is comparable to assay methods based on radioactively labelled carbodiimides.

EXPERIMENTAL

Spray reagent was prepared by dissolving 500 mg, *N,N'*-dimethylbarbituric acid in 10 ml pyridine–water (9:1). The TLC plate was sprayed intensively until transparency or dipped briefly into the reagent. Without drying or heating, the moistened plate was kept in a closed petri dish for 5–10 min. The appearance of blue or purple

spots indicates the presence of as little as 1 nmol of carbodiimide. The background remains absolutely colourless.

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