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Note

Gas chromatographic determination of dicyclohexylurea in the active esters of amino acids

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The reaction between the carboxyl group of amino acids or peptides and halo- or nitrophenols to form the so called active esters is one of the most important reactions of modern peptide chemistry. As the condensing reagent of this reaction is dicyclohexylcarbodiimide, an equivalent quantity of dicyclohexylurea (DCU) is also formed. Because of its unfavourable crystallization properties the removal of the latter from active esters is not an easy task, therefore the quantitative determination of DCU, as the contaminant of active esters, in amounts down to 0.1% is one of the important tasks of peptide analysis.

As DCU is titrimetrically and spectrophotometrically inactive and its –CO–NH– grouping which may serve as the basis for colorimetric measurements can be found in peptides and protected amino acids as well, we decided to use gas chromatography to solve the above problem.

No data have been found in the literature regarding the gas chromatography of DCU. Reiser[1] described the gas–liquid chromatographic separation of some alkyl-substituted urea derivatives on glass beads covered with 0.5% Carbowax 20M but no groups larger than butyl were investigated. Recently Evans[2] chromatographed some N,N'-disubstituted urea derivatives as the trifluoroacetates on Diatomite C covered with 10% PEGA.

As the derivatization of small amounts of DCU in the presence of large quantities of amino acids or peptides cannot be carried out DCU was chromatographed without derivatization.

After having failed to obtain suitable chromatograms on various stationary phases it has been found that this non-volatile material (m.p. 227°) can only be chromatographed on non-polar methylsilicone phases; JXR on Chromosorb W HP has been selected. Using this column at 190° DCU gives a symmetrical peak (retention time 3.0 min) sufficiently separated from the solvent peak and that of docosane (5.1 min) used as the internal standard.

A linear relationship has been found between the ratio of the peak areas and the amount of DCU within the concentration range 0.1–2.5 μg DCU/μl. The relative molar response of DCU (relative to docosane) is 0.22. As about 50 μg of the active ester is injected on to the column this means that 0.2–5% DCU contamination can smoothly be determined with the described method. This was confirmed by the evaluation of serial tests on model mixtures. A relative standard deviation of $\pm$ 1.8%

was found when a model mixture containing 2.0% DCU in *tert.*-butoxycarbonyl glycine pentachlorophenyl ester was investigated.

The materials investigated included pentachlorophenyl, pentafluorpohenyl and *p*-nitrophenyl esters of amino acids such as glutamine, asparagine, histidine, glycine, alanine, phenylalanine, nitroarginine as well as some peptides with benzyloxycarbonyl or *tert.*-butoxycarbonyl protecting groups at the amino terminals. It should be noted that these materials are not chromatographed under the conditions described and no thermal decomposition leading to products interfering with the gas chromatographic determination has been found either. This naturally means that relatively large quantities of these materials are accumulated at the injector zone of the column. It is advisable therefore to replace the column load in this zone by a fresh one after about 10 injections.

EXPERIMENTAL

A Hewlett-Packard 7620 gas chromatograph with a flame ionization detector was used in this study. The chromatographic conditions were as follows: Column, glass tube, 4 ft. × 2 mm I D., packed with 3% JXR on Chromosorb W HP, 90–100 mesh; Oven temperature, 190°; Vaporizer zone and detector temperature, 260°; Carrier gas (nitrogen) flow-rate, 35 ml/min.

Procedure

A 0.05-g amount of the material to be investigated is dissolved in 1 ml of ethanol–chloroform (1:1) containing 0.4 mg of docosane and 1 μl of this solution is injected on to the column. The DCU content is calculated from the ratio of the peak areas by means of a calibration graph.

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