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Note

Thin-layer chromatographic separation of uracil and dihydrouracil

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The first reaction of uracil degradation is catalysed by uracil reductase and dihydrouracil dehydrogenase, respectively (E.C. 1.3.1.1, E.C. 1.3.1.2)*. Generally its activity is measured spectrophotometrically by oxidation of NADPH or NADH[1] and is visualized with nitroblue tetrazolium[2]. However, in organisms with low activity of uracil reductase, high sensitivity of radiometric methods is required. Quantitative separation of uracil and dihydrouracil is a prerequisite to this determination. Because of restricted resolution of pyrimidines on Sephadex G-10 in gel chromatography[3], on Dowex-1 in ion-exchange chromatography[4] and in paper chromatography[5], separation of uracil and dihydrouracil or thymine and dihydrothymine by thin-layer chromatography (TLC) seems to be the most valuable method[6]. Unfortunately, use of solvent systems described previously[6,7] results in incomplete separation of uracil, dihydrouracil and their degradation product β-ureidopropionic acid, and thymine and dihydrothymine, respectively.

Therefore, adsorption chromatography on Kieselgel D (VEB Chemiewerk, Greiz-Dölau, G.D.R.) was used and modified to give high resolution of uracil and dihydrouracil. The solvent system chloroform–methanol–acetic acid (100:5:1)[6] was used in our studies. TLC plates (20 × 20 cm, 0.5 mm thick) were prepared with Kieselgel D (VEB Chemiewerk, Greiz-Dölau, G.D.R.) activated (10 min at 110°). Separation was performed continuously in an open sandwich chamber (3 mm distance of the plates) at a low temperature (4°) for 12 h. For evaporation of the solvents, chromatographic paper 4 × 20 cm, Filtrak FN 7 (VEB Spezialpapier, Niederschlag, G.D.R.) was placed on the upper edge of adhering adsorbent. [2-^{14}C]Uracil (UVVVR, Prague, Czechoslovakia), [2-^{14}C]dihydrouracil (Rotop, Dresden, G.D.R.), [ureido-^{14}C]propionic acid (prepared from [2-^{14}C]dihydrouracil by the method of Fink *et al.*[8]) and an unlabelled tracer were applied on TLC plates with glass capillaries giving extremely small starting points.

In Fig. 1 complete separation of uracil, dihydrouracil and β-ureidopropionic acid is demonstrated by their scanner profile. Contrary to earlier TLC of pyrimidines and dihydropyrimidines, the use of continuous conditions with the sandwich tech-

* In accordance with Hallock and Yamada[2] the name uracil reductase will be used for the enzyme catalyzing uracil/thymine → dihydrouracil/dihydrothymine reactions, and the name dihydrouracil dehydrogenase for the enzyme catalyzing the reactions dihydrouracil/dihydrothymine → uracil/thymine.

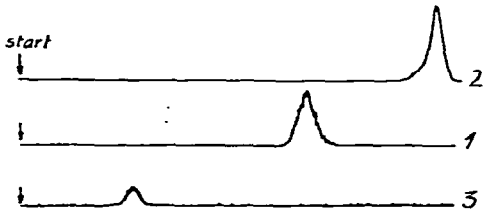

Fig. 1. Separation of uracil (1), dihydrouracil (2) and β-ureidopropionic acid (3) by TLC with Kieselgel D plates in the solvent system chloroform–methanol–acetic acid (100:5:1) at 4° (continuous sandwich conditions, 12 h).

nique and low temperature is of great value. Running time and temperature, determine the separation distance of uracil and dihydrouracil.

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