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The separation and detection of methionine and its α-keto and α-hydroxy analogues on thin-layer chromatograms

The study of the biological interconversion of methionine, 2-keto-4-methylthiobutyric acid (methionine keto analogue, MKA), and 2-hydroxy-4-methylthiobutyric acid (methionine hydroxy analogue, MHA) required a method for their chromatographic separation and qualitative detection. The method described herein fulfills the need for such a system.

Standard solutions of methionine, MKA, MHA, and a mixture of the compounds were spotted on thin-layer chromatograms (silica gel; Eastman Chromagram Sheet No. 6061) and dried for 10 min in an oven at 85°. The chromatogram was developed, ascendingly, using 1-butanol–acetic acid (glacial)–water (170:25:5) until the solvent front had migrated 15 cm past the origin (about 2 h) after which the chromatogram was air dried. Methionine was detected by spraying with a 1% acetone solution of ninhydrin followed by heating at 85°. After circling the methionine spot, MKA and MHA were detected by a modification of the method of McCARTHY AND SULLIVAN[1]. The chromatogram was sprayed with a 1% aqueous solution of glycine immediately followed by spraying with a 14.3 *N* NaOH solution. After 30 sec the excess fluid was removed by patting with filter paper followed by spraying with a 10% aqueous solution of sodium nitroprusside. The MKA immediately appeared as a deep purple spot. The chromatogram was again patted dry and the location of the MKA was noted. The chromatogram was then heated for 5 min at 85° during which the MKA spot disappeared. Spraying with an acid mixture (12 *N* HCl–85% H_3PO_4, 9:1) resulted in the MHA appearing as a light violet-red spot and after about 5 min the MKA area reappeared as a light blue area, with all spots fading within 30 min. To establish that the MKA spot was actually the keto analogue, a separate chromatogram was sprayed with 0.4% 2,4-dinitrophenylhydrazine (in 2 *N* HCl) and 10% NaOH according to the method of NEWCOMBE AND REID[2]. The yellow spot that developed coincided exactly with the deep purple and light blue spots noted as belonging to the MKA in the former method.

The 2-keto-4-methylthiobutyric acid was a gift of Dr. MINORU NAKANO, Department of Biochemistry, School of Medicine, Gunma University, Maebashi, Gunma,

TABLE I

THE R_F VALUES AND MINIMUM DETECTABLE QUANTITIES (MDQ) OF METHIONINE, 2-KETO-4-METHYLTHIOBUTYRIC ACID, AND 2-HYDROXY-4-METHYLTHIOBUTYRIC ACID ON SILICA GEL THIN-LAYER CHROMATOGRAMS

Developing solvent: 1-butanol–glacial acetic acid–water (170:25:5). Detection methods are given in the text.

Compound	R_F	*MDQ (μmoles)*
Methionine	0.35	0.001–0.01
2-Keto-4-methylthiobutyric acid	0.68	0.1 (purple) 0.05 –0.1 (l. blue)
2-Hydroxy-4-methylthiobutyric acid	0.86	0.1 –0.5

Japan. The 2-hydroxy-4-methylthiobutyric acid is a commercial product of the Monsanto Company, St. Louis, Mo. 63166, U.S.A. and was donated through the courtesy of Mr. N. L. REDING.

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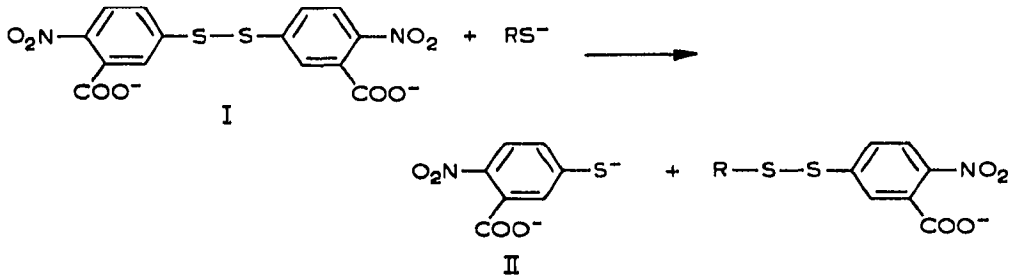
Chromatographic detection of thiols, disulfides, and thioesters with 5,5′-dithiobis(2-nitrobenzoic acid)

Few reagents are available for the detection of sulfhydryl containing compounds on chromatograms. Sodium nitroprusside[1], platinic iodide[2], or various quinones[3], which are not specific for sulfhydryl, have been used. Recently GRASSETTI AND MURRAY[4] reported the use of 2,2′-dithiobis(5-nitropyridine) as a selective reagent for the detection of thiols.

We have used alcohol–buffer solutions of 5,5′-dithiobis(2-nitrobenzoic acid)[5] (I, DTNB, ELLMAN reagent) as a convenient and sensitive spray reagent for the visualization of thiols on chromatograms as yellow spots*. The reagent reacts specifically with sulfhydryl groups by a disulfide exchange reaction to give the yellow thioanion (II) of 2-nitro-5-mercaptobenzoate[5].



I

II

We have extended this method to the chromatographic detection of disulfides and thioesters, which are both easily converted to their thiol derivatives: the former by reduction with sodium borohydride and the latter by alkaline hydrolysis.

* GRASSETTI AND MURRAY[4] reported that DTNB "does not appear to be suited as a spray reagent in organic media" for the chromatographic detection of thiols.