

A new colorimetric test for detecting sulfur-containing amino acids

Sulfur-containing amino acids and particularly the S-alkyl cysteines, methionine, and related amino acids are frequently detected colorimetrically by the iodoplatinate procedure¹ or by the iodine azide method². These reagents do not, however, distinguish sulfides from sulfoxides and frequently the latter compounds give weak, indecisive responses. The present study arose from the need for a satisfactory colorimetric reagent for detecting "cycloalliin"³ (3-carboxy-5-methyl-1,4-thiazane-1-oxide) on paper chromatograms. This amino acid at low concentration gives virtually no color with ninhydrin or with isatin and has only a weak bleaching action on the iodoplatinate reagent.

We have found that when paper chromatograms are sprayed with FEIGL'S⁴ reagent for secondary amines (aqueous sodium nitroprusside-acetaldehyde solutions) and then fumed with hydrochloric acid, S-substituted cysteines and methionine yield blue colors and the corresponding sulfoxides give pale violet colors. Both cycloalliin and reduced cycloalliin (3-carboxy-5-methyl-1,4-thiazane) respond to this test. Surprisingly, neither of these compounds gives a blue color before acid treatment, although the original Feigl test is very sensitive for such other cyclic secondary amino acids as proline and pipercolic acid. 3-Carboxy-1,4-thiazane, a homologue (without the methyl group)* of reduced cycloalliin, does yield a blue color with the Feigl test for secondary amines, and also a blue on subsequent treatment with hydrochloric acid, in agreement with our other results. In some cases, N-acylated sulfide or sulfoxide amino acids will respond to the test. As shown in Table I, two N-acetyl derivatives responded, while two N-benzoyl and one carbobenzyloxy derivative gave negative results.

The test is sufficiently sensitive to detect cycloalliin, reduced cycloalliin, S-methyl-L-cysteine and the corresponding sulfoxides on paper chromatograms at levels of 3 γ per spot. Table I lists compounds that give positive responses to the test. Cysteine and cystine yield pale greenish-blue and muddy blue colors, respectively. Those amino acids that do not contain sulfur generally yield muddy green or grey colors which can easily be distinguished from the light blue or lavender of the sulfide or sulfoxide amino acids.

Experimental

Paper. Schleicher and Schüll 589 Blue Ribbon and Whatman No. 1 papers were used**.

Reagents. 2 % w/v sodium bicarbonate in 30 % aqueous ethanol. 2 % w/v aqueous sodium nitroprusside. 20 % v/v acetaldehyde in aqueous solution.

The nitroprusside and acetaldehyde solutions should be kept under refrigeration and mixed in equal proportions before use. The mixed reagent is usually effective for a week when stored in the cold, but best results are obtained with freshly mixed solutions.

Procedure. The developed paper chromatogram, after air-drying, is sprayed with the bicarbonate solution and then dried again. The paper is then sprayed with the mixed acetaldehyde-nitroprusside reagent (proline is a good indicator since it

* Synthesis to be published.

** Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

TABLE I
 COLOR REACTION OF SOME S-CONTAINING AMINO ACIDS WITH
 SODIUM NITROPRUSSIDE-ACETALDEHYDE REAGENT

<i>Amino acid</i>	<i>Color</i>
S-Methyl-L-cysteine	Blue
S-Methyl-L-cysteine sulfoxide	Lavender*
S- <i>n</i> -Propyl-L-cysteine	Blue
S- <i>n</i> -Propyl-L-cysteine sulfoxide	Lavender
S-Allyl-L-cysteine	Blue
S-Allyl-L-cysteine sulfoxide	Lavender
S-(β -Hydroxyethyl)-L-cysteine	Blue
S-(β -Hydroxyethyl)-N-acetyl-L-cysteine methyl ester	Blue
S-(β -Hydroxyethyl)-N-acetyl-L-cysteine methyl ester sulfoxide	Lavender
N-Benzoyl-S- <i>n</i> -propyl-L-cysteine sulfoxide	—
Dibenzoyl-mesolanthionine	—
Dicarbobenzyloxy-mesolanthionine	—
Djenkolic acid	Blue
Mesolanthionine	Blue
L-Allocystathionine	Blue
Cycloalliin (sulfoxide)	Violet
Reduced cycloalliin	Blue
3-Carboxy-1,4-thiazane	Blue
Methionine	Blue
Methionine sulfoxide	Blue-violet
Methionine sulfone	Blue-violet

* No significant difference was observed between the *dextro* and *levorotatory* S-methyl-L-cysteine sulfoxides.

invariably gives a strong blue color at this stage), air-dried, and fumed with hydrochloric acid in a suitable chamber for 15–30 sec for sizes of paper up to 24 × 24 cm or 45–60 sec for larger chromatograms. The paper is hung in a fume hood and the colors noted from 15 min to an hour after fuming. The color produced by cycloalliin is stable and often becomes more intense after 24 h, but for most of the compounds tested the color gradually fades after several hours.

Western Regional Research Laboratory,
 Western Utilization Research and Development Division,
 Agricultural Research Service, U.S. Department of Agriculture,
 Albany, Calif. (U.S.A.)

JOHN F. CARSON
 FRANCIS F. WONG

¹ H. M. WINEGARD, G. TOENIES AND R. J. BLOCK, *Science*, 108 (1948) 506.

² E. CHARGAFF, C. LEVINE AND C. GREEN, *J. Biol. Chem.*, 175 (1948) 67.

³ A. I. VIRTANEN AND E. J. MATIKKALA, *Acta Chem. Scand.*, 13 (1959) 623.

⁴ F. FEIGL, *Spot Tests in Organic Analysis*, 6th Ed., Elsevier, Amsterdam, 1960, p. 273.

Received March 21st, 1963