A NEW COLOR TEST FOR TRYPTOPHAN AND RELATED COMPOUNDS

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It has been reported in a preliminary communication (1) that under certain conditions, at room temperature, perchloric acid converts tryptophan to a yellowish green fluorescent compound. Fluorescence was found to be particularly strong in ultraviolet light. It has now been found that a small amount of bichromate very considerably increases the sensitivity of this reaction and that the test may also be applied to certain compounds which are related to tryptophan. This reaction, however, is not given by any amino acid other than tryptophan. Tryptophan may thus be readily identified in untreated proteins.

EXPERIMENTAL

Solutions

Perchloric Acid—The usual commercial product, c.p., 70 to 72 per cent.

Dichromate Solution—10 mg. of potassium dichromate (c.p.) in 100 cc. of distilled water.

Ferric Chloride Solution—1 gm. of ferric chloride (FeCl₃·6H₂O, c.p.) in 100 cc. of distilled water.

Color Test

0.5 cc. of water containing 0.5 mg. of tryptophan or about 10 mg. of albumin (egg powder) or any other tryptophan-containing substance is placed in a test-tube. It is not necessary that the material to be tested shall be in solution. If certain indole derivatives are to be tested, they may be dissolved in ethyl alcohol. 3 cc. of perchloric acid and 0.1 cc. of bichromate solution are added and the contents of the tube are well mixed. A quite stable, intensely greenish yellow color forms immediately. Upon the addition of 0.1 cc. of 1 per cent ferric chloride solution, the greenish yellow color becomes deep reddish orange within a few minutes. For the detection of minute amounts of tryptophan, the addition of ferric chloride solution should be omitted and ultraviolet light should be employed.

In Table I are shown typical color reactions as given by tryptophan and a few compounds closely related to tryptophan. All substances, however,
show much less intense fluorescence when treated with perchloric acid and bichromate solution than does tryptophan. Distinct color differences are displayed by the various compounds after the addition of the ferric chloride solution to the perchloric acid-bichromate reaction mixture. The following tryptophan-containing proteins gave the color reaction: casein, albumin (egg powder), human blood serum, pepsin, and crystalline soy bean trypsin inhibitor. Gelatin (Difco) and silk (U. S. P. type A-O, Product 480, Davis and Geck) did not give the test. A 10 mg. sample of zein in 80 per cent ethyl alcohol showed only very slight fluorescence, indicating the presence of only a trace of tryptophan.

**TABLE I**

| **Color Reactions with Perchloric Acid-Dichromate and Ferric Chloride** |
|-----------------|-----------------|-----------------|
| **Tryptophan**  | Greenish yellow | **Color after**  |
| **Skatole**     | Light yellow    | **subsequent**  |
| **Indole**      | " " (almost    | **addition**    |
| " acetic acid.  | colorless)      | of ferric chloride | Brown |

**SUMMARY**

Tryptophan and related compounds give typical color reactions with the perchloric acid test. The amino acids, glycine, alanine, leucine, isoleucine, valine, phenylalanine, tyrosine, cysteine, cystine, methionine, threonine, proline, hydroxyproline, histidine, arginine, lysine, serine, aspartic acid, glutamic acid, and p-aminobenzoic acid do not give the reaction.

Although certain phenols react with ferric chloride (as in the second part of this test), perchloric acid and dichromate do not give a color reaction with small amounts of phenols and the phenols do not form fluorescent compounds under similar conditions.

Cohen (2) made the remarkable observation that, when carbohydrates are heated together with tryptophan for 10 minutes at 100° in 30 per cent perchloric acid, colored condensation products form. In his reaction boiling is an essential factor. The green fluorescent substances described herein, however, form immediately at room temperature and carbohydrates do not interact.

**BIBLIOGRAPHY**

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