COLORIMETRIC ESTIMATION OF INDOLEACETIC ACID

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(WITH ONE FIGURE)

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The wide use of the auxin, indoleacetic acid, in physiological and biochemical experiments has promoted interest in methods for its colorimetric estimation. MITCHELL and BRUNSTETTER (**1**) have proposed both the nitrite and the ferric chloride–sulphuric acid tests for the quantitative estimation of indoleacetic acid (IAA) in aqueous solutions, basing their suggested procedures upon a study of optimal reaction conditions for these two reagents. According to them, the nitrite method is sensitive to 10 μg. IAA/ml. and develops a red color that is stable after two hours. In several attempts to duplicate their nitrite method using solutions of IAA varying from 20 to 45 μg./ml., we could not obtain a stable red color with IAA at the two hours proposed, or at any other time. A faint pink develops almost immediately which rapidly fades to orange or yellow, depending on IAA concentrations, within $\frac{1}{2}$ hour. If the concentration of nitrite is reduced, the red color becomes sufficiently persistent to be read. Indole likewise gives a strong, relatively stable, red color in this test (cf. table II)—a reaction which is sometimes used as a qualitative test for indole (Nitroso-Indole reaction).

TANG and BONNER (**2**) have modified the ferric chloride–sulphuric acid method for IAA, combining the iron and sulphuric acid as a single reagent to yield improved sensitivity. However, the color produced is also unstable, rapidly developing and then fading. We have found, as have these workers, that the fading color can be practically dealt with by adopting a standard time between addition of reagent and reading of absorbancy or transmittance.

Both of the methods discussed above possess disadvantages, lacking either specificity, sensitivity, or stability of color complex formed. During a study of the inactivation of IAA in aqueous solutions, it was frequently necessary to assay at one time many samples where the IAA concentrations were low, or where the degree of significance of small differences in concentrations between experimental units required evaluation. Hence, we considered it desirable to re-examine the ferric chloride–sulphuric acid procedure. Several alterations have been made which produce a more stable color, of increased specificity, which changes in density more rapidly with variation in IAA concentration.

1. The procedure of Tang and Bonner can be improved somewhat by reading at 15 minutes after addition of reagent (instead of 30 minutes as they suggest), since the transient color reaches a maximum at the former time. Maximum absorption was found to occur at 530 mμ.

2. Color density and stability as a function of amount of Tang and Bonner reagent added to varying concentrations of IAA were then determined. It was found that a color of maximum density was obtained when the volume ratio of reagent to IAA solution was 1.5 : 1. Moreover, at this ratio, the color becomes stable for several hours 50 to 80 minutes after addition of reagent. With this modification, the $\lambda_{max.}$ is 525 mμ. The increase in sensitivity and stability attained is offset, in part, by increased reactivity of several other indole compounds (table II).

It was considered that a trace of hydrogen peroxide might contribute to an oxidative reaction, hastening the color development, and perhaps increasing its intensity. However, final solution concentrations of hydrogen peroxide from 2×10^{-2} to 2×10^{-6} M resulted in decreased color density and promoted color instability, though the attainment of maximum color was hastened. Lower concentrations of hydrogen peroxide were without appreciable effect.

3. When perchloric acid was substituted for sulphuric acid in the Tang and Bonner reagent, color intensity was improved. By varying the volume

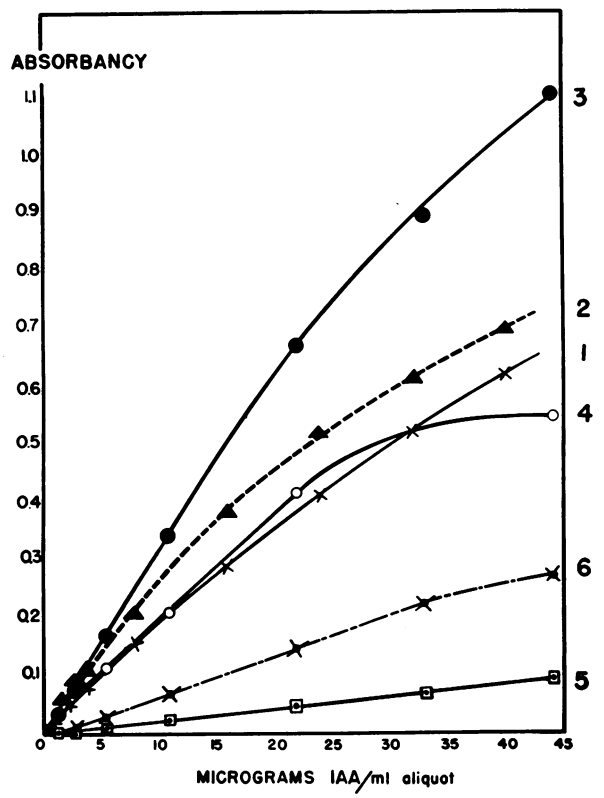

FIG. 1. Concentration-absorbancy curves for indoleacetic acid. Curve numbers correspond to procedures given in table I. Absorbancies determined by Coleman Mod. 14 spectrophotometer, using the shielded cuvette carrier for small volumes.

TABLE I

REAGENTS AND PROCEDURES USED FOR THE COLORIMETRIC ESTIMATION OF INDOLE-3-ACETIC ACID

Reagent	Ml. IAA solution	Ml. reagent	λ_{max} mμ	Remarks
1. Fe-H_2SO_4 1.0 ml. 0.5 m $FeCl_3$ 50 ml. Dist. H_2O 30 ml. H_2SO_4, Sp. Gr. 1.84	1.0	4.0	530	Read at 15′
2. Fe-H_2SO_4 (modified) components same as above	1.0	1.5	525	Read after 75′
3. Fe-$HClO_4$ 1.0 ml. 0.5 M $FeCl_3$ 50 ml. 35% $HClO_4$	1.0	2.0	530	Read after 25′
4. Co-$HClO_4$ 1.0 ml. 0.5 M $Co_2F_6 \cdot 7H_2O$ 50 ml. 35% $HClO_4$	1.0	2.0	528	Boil 10′, cool and read
5. Hg-$HClO_4$ 1.0 ml. 0.5 M $Hg(NO_3)_2$ 50 ml. 35% $HClO_4$	1.0	2.0	555	Read at 15′
6. KNO_2-HNO_3 .05 ml. 2.5% Gum Arabic .025 ml. 0.5% KNO_2 .04 ml. conc. HNO_3	5.0	Reagent components added individually to IAA aliquot	527	Read at 8′ after add'n of HNO_3

and concentration of reagent added to fixed volumes containing varying amounts of IAA, the optimal proportions were found to be 2 parts 0.01 M $FeCl_3$ in 35% $HClO_4$ to 1 part IAA solution. Color density reaches its maximum at approximately 20 to 25 minutes and remains virtually constant for at least three hours.

Other metallic salts (1 part 0.5 M to 50 parts 35% $HClO_4$) were tested with IAA: $SrCl_2$, $TiSO_4$, $SnCl_2$, $Cu(NO_3)_2$, $Ce(HSO_4)_4$, CsCl, $NiCl_2$, $UO_2(NO_3)_2$, $CdCl_2$, $Hg(NO_3)_2$, and CoF_6. Of these, only Co and Hg gave appreciable color reactions, but neither was as satisfactory as the ferric chloride–perchloric acid procedure given above.

In figure 1 are given the concentration-absorbancy curves of those modifications discussed, and the procedures followed are detailed in table I. The relative absorbancy of various other 3-substituted indoles in these methods are given in table II. It can be seen that the $FeCl_3$–$HClO_4$ reagent is not only more sensitive within the IAA concentration range studied, but at the same time shows least interference from other indole compounds. We have, therefore, adopted the following colorimetric procedure for routine estimations of IAA in aqueous solution in concentrations of 0.2 to 45 μg./ml.:

1. To a 1.0-ml. aliquot add 2.0 ml. of $FeCl_3$–$HClO_4$ reagent
2. Read after 25′ at 530 mμ.

   Reagent: 1.0 ml. of 0.5 M $FeCl_3$
   50 ml. 35% $HClO_4$

TABLE II

PER CENT. OF IAA ABSORBANCE DEVELOPED BY VARIOUS INDOLE COMPOUNDS EQUIMOLAR ALIQUOTS (0.125 MILLIMOLAR) TREATED ACCORDING TO THE PROCEDURES OF TABLE I

	1	2	3	4	5	6
	$Fe\text{-}H_2SO_4$	$Fe\text{-}H_2SO_4$ (modified)	$Fe\text{-}HClO_4$	$Co\text{-}HClO_4$	$Hg\text{-}HClO_4$	$KNO_2\text{-}HNO_3$
Indole	12.4	20.6	9.86	16.9	25.0	325.
Skatole	13.4	26.0	10.9	40.8	25.0	24.2
Indole-3-aldehyde .	3.22	3.65	0.86	23.1	8.35	1.96
Indole-3-carboxylic acid	6.20	9.90	4.03	27.0	16.6	10.4
Indole-3-propionic acid	0.74	0.52	0.00	0.96	6.25	8.50
Indole-3-butyric acid	14.4	26.9	11.9	28.9	2.85	11.1
Tryptophan.	0.74	0.42	0.00	1.91	4.17	5.88

Since Beer's law is not followed at high concentrations of IAA, absorbancies obtained are converted to IAA concentrations by a standard curve. We have found that such a curve does not vary appreciably when rechecked with reagent stored in the light at room temperature for over three months. The method has the advantage of being relatively sensitive and is of higher specificity than the methods heretofore used. The stability of the color formed allows a large number of determinations to be made simultaneously without adherence to a fixed time schedule.

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