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Qualitative Method for Partial Characterization of Indole Derivatives[1]

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The ability of indole and tryptophan to form colored products by reacting with *p*-dimethylaminobenzaldehyde (PDAB) has been known for over 50 years. This property was applied to the detection and later to the quantitative determination of these indole compounds (1–6). While numerous attempts have been made to identify the colored products of these reactions (7–13), no serious effort has been made to establish reaction conditions which might help to identify other indole derivatives.

Our study of these color reactions indicated a rapid condensation between the aldehyde group of PDAB and the pyrrole moiety of the indole derivative preferentially at the C-3 carbon; a much slower reaction at C-2 carbon occurred when C-3 was blocked by a substituent (14).

The reactions studied indicated that the rates and conditions necessary for color formation could be used as a first guide to the determination of a structure of an indole derivative. In addition this information could be utilized for the quantitative determination of certain indole derivatives in mixtures without significant mutual interference.

MATERIALS AND METHODS

Reagents

PDAB solution: 35 mg of *p*-dimethylaminobenzaldehyde per milliliter of 3 *N* H_2SO_4.

30 *N* H_2SO_4.

Nitrite solution: 0.05% $NaNO_2$ in water.

[1] A portion of this work was taken from a Ph. D. dissertation submitted to the Graduate Division, University of California, Los Angeles.

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Procedure

Reaction I: 1 ml of PDAB solution was added to 5-ml aqueous samples containing the indole derivatives.[3] Ten minutes was allowed for color development, after which the absorbancy of the solution was measured at 550 mμ[4]). Solutions which did not develop color, or did not reach maximum, within this time were kept for longer periods for observation. Solutions were designated *Negative* when color did not form even after 72 hr. Solutions were designated as *Slow*-reacting when maximal color was not attained in 10 min, and as *Fast*-reacting when full color development was achieved within 10 min.

Reaction II: Following 30 min of Reaction I, the solutions were chilled by immersing tubes in ice water. In subdued light, 3 ml of 30 *N* H_2SO_4 was added with thorough mixing and the solutions were kept in the dark for 1 hr at room temperature.[5] Color was developed by: (*a*) addition of 0.1 ml of nitrite solution with quick thorough mixing and leaving for 30 min in the dark: *or by* (*b*) exposure to ordinary fluorescent laboratory light for 72 hr. Absorbancy was measured at 580 mμ.

Reaction II was designated as *Positive* when maximal color was attained with $NaNO_2$ within 30 min or by exposure to light for 72 hr, as *Weakly Positive* when weak color (less than one-third as compared to similar concentrations of tryptophan) was attained with $NaNO_2$ in 30 min or by exposure to light for 72 hr, and as *Negative* when color did not form under the above conditions.

RESULTS

Tables 1 and 2 indicate that indole derivatives could be divided into three groups according to their reactivity under conditions of Reaction I:

Reaction I

Fast— no substituents on C-3 and no electron-withdrawing groups on the nucleus.

Slow— no substituents on C-2 but containing a substituent on C-3 (this substituent not containing a free amino group); no electron-withdrawing group on the nucleus.

Negative—(*1*) C-2 unsubstituted; C-3 substituted, with a free amino group in the side chain,
(*2*) an electron-attracting group on the nucleus,
(*3*) substituents on both C-2 and C-3.

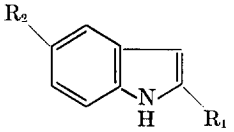
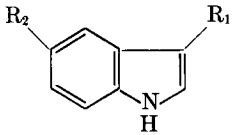
[3] Usually 0.3–0.6 μmole of an indole derivative was present.

[4] All readings except when otherwise specified were obtained with a Beckman model B spectrophotometer.

[5] Colors developed during Reaction I, faded.

TABLE 1

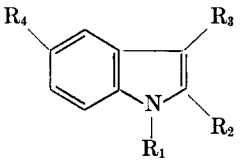
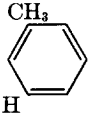
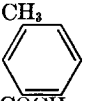
EFFECT OF SUBSTITUENTS ON REACTIVITY OF INDOLES

		Reaction I			Reaction II		
						Weakly	
R_1	R_2	Fast	Slow	Neg.	Pos.	pos.	Neg.
							
H	H	X					X[a]
CH_3	H	X					X
	H	X					X
H	CH_3	X				X	
H	OH	X				X	
H	OCH_3	X				X	
							
CH_3	H		X		X		
CH_2COOH	H		X		X		
CH_2COOH	OH		X		X		
CH_2CH_2COOH	H		X		X		
$CH_2CH_2CH_2COOH$	H		X		X		
$CH_2CHOHCOOH$	H		X		X		
$CH_2CH_2NHCOCH_3$	H		X		X		
$CH_2CH(NHCOCH_3)COOH$	H		X		X		
$CH_2CHNHCOCH_2CH_2NH_2$ COOH	H		X		X		
CH_2CHNHC(=O)–C_6H_4–NO_2 COOH	H		X		X		
$CH_2CH_2NH_2$	H			X[b]	X		
$CH_2CH_2NH_2$	CH_3		X		X		
$CH_2CH(NH_2)C_6H_5$	H			X[b]	X		
$CH_2CH(NH_2)COOH$	H			X[b]	X		
$CH_2CH(NH_2)COOH$	OH			X[b]	X		
$CH_2CH(NH_2)COOH$	CH_3			X	X		
$CH_2CH(NH_2)COOH$	F			X	X		
—$CH_2CH(NH_2)CONHCHCH_2$–C_6H_4–OH COOH	OH			X	X		
—$CH_2CH(NH_2)CH_2CH_3$	H			X	X		

[a] While no color formed with nitrite, exposure to light for 24 hr yielded a weak gray color.

[b] Even after weeks of standing.

TABLE 2
EFFECT OF SUBSTITUENTS ON REACTIVITY OF INDOLES

				Reaction I	Reaction II	
R_1	R_2	R_3	R_4	Neg.	Weakly pos.	Neg.
						
H	CH_3	CH_3	H	X		X
H			H	X		X
$COCH_3$	H	$COCH_3$	H	X		X
H	$COOC_2H_5$	H	H	X		X
H	H	$COCH_3$	H	X		X
H	H	CHO	H	X		X
H	H	$CH_2N(CH_3)_2$	H	X		X[a]
H	H	$CH_2N(CH_3)_2$	OCH_3	X	X	—

[a] While no color formed with nitrite, exposure to light for 48 hr yielded a weak purple color.

Compounds of the *Fast*-reacting group yielded color (pink or purple) almost instantaneously, achieving close to maximal color development within 2 min. On the other hand, derivatives of the *Slow*-reacting group (except for skatole which attained about 5% of its maximal color in 2 min) remained colorless for periods of 10 min (indolebutyric acid) to several days (indoleacetic acid). Reactivity within this group varied widely; the rates in decreasing order were: skatole >> indolebutyric acid > indolepropionic acid > *N*-acetyltryptamine > *N*-acetyltryptophan > indolelactic acid > β-alanyltryptophan > indoleacetic acid. While their rates of reaction varied widely, the colors formed had similar spectra[6] (different from *Fast*-reacting derivatives): skatole (λ_{max} = 580, 618 mμ); indolebutyric acid (λ_{max} = 580, 625 mμ); indolepropionic acid (λ_{max} = 578, 620 mμ); *N*-acetyltryptophan (λ_{max} = 575, 630 [plateau] mμ) and indoleacetic acid (λ_{max} = 580, 625 mμ). Initially the 580 peak is the prominent peak and only a plateau is observed in the 610–630 mμ region. On standing (to allow further color development) the plateau develops into a peak of intensity equal to that at 580 mμ.

The low reactivity of indoleacetic acid as compared to indolepropionic and indolebutyric acids is presumably due to the deactivating effect of the carboxyl group. Conversely, an activating group (OH) increased the reactivity of 5-hydroxyindoleacetic acid. A similar activation of the

[6] Obtained with a Cary recording spectrophotometer.

indole nucleus was observed with 5-methyltryptamine (Table 1); while other compounds with a free amino group in the side chain remained colorless for weeks, introduction of a methyl group altered the reactivity so that in 24 hr a significant color developed, placing 5-methyltryptamine in the *Slow*-reacting group. A similar substitution of tryptophan did not facilitate color formation; both 5-hydroxy and 5-methyltryptophan are in the *Negative* group (Table 1).

It is interesting to note that compounds with a side chain at C-3 that contains a free amino group (e.g., tryptophan) remain colorless; here the free amino group entirely alters the reaction and color forms only in Reaction II. Similar compounds lacking the NH_2 group (indolepropionic acid, indolelactic acid) or with a substituted NH_2 (*N*-acetyltryptophan) reacted under Reaction I conditions and fitted into the *Slow*-reacting group.

While Reaction I permitted a definite classification into groups via the rates and reactivity of the different indole derivatives, Reaction II was not suited for such a classification. Indole derivatives under conditions of Reaction II were classified as *Positive, Weakly Positive,* and *Negative.* Though a definite distinction could be drawn between Reaction II *Positive* and *Negative* compounds, the *Weakly Positive* derivatives required a point of reference. Tryptophan was chosen as a reference because of its color stability and rapidity of achievement of maximal color (24-hr exposure to light).

Reaction II

(*a*) *Positive*— a C-3 substituent; no substituent at C-2 and no electron-attracting group on the nucleus.

(*b*) *Weakly Positive*—a C-2 substituent; no substituent at C-3 and an activating group at C-5.

(*c*) *Negative*—
- (*1*) an electron-withdrawing group on the nucleus,
- (*2*) substituents at both C-2 and C-3,
- (*3*) a C-2 substituent but no C-3 substituent; or no substituents in either position.

In attempting to achieve reproducible intensity of colors, nitrite concentration was found to be critical and had to be established for each compound used. It was observed that stable and more intense colors could be obtained by light catalysis especially in the case of compounds lacking an amino group (skatole, and indoleacetic and indolepropionic acids); the color of these compounds was unstable when developed by nitrite. The absorption spectra of colors resulting from treatment with

nitrite or from light catalysis were similar for all compounds giving a *Positive* Reaction II; one peak between 590 and 620 mμ was observed.

DISCUSSION

The results presented indicate that indole derivatives could be classified into several groups, according to their reactivity with PDAB under various conditions. The different conditions have been designated as: Reaction I—0.5 *N* H_2SO_4; Reaction II—approximately 11 *N* H_2SO_4 in the dark followed by the addition of nitrite or exposure to light.

It was observed that the location of the substituent, i.e., the availability of the pyrrole moiety for condensation with PDAB, was the prime factor in determining the reactivity and the shade of the color formed. These reactions would permit a rough guess as to the location of a substituent on the pyrrole moiety and at times would indicate certain features in the nature of the substituent.

Reaction I conditions provide information as to the availability of a free site on the pyrrole moiety of the indole nucleus: a *Fast* reaction indicates a free C-3; a *Slow* reaction, a free C-2 but a substituted C-3. A *Negative* reaction may indicate a variety of factors. Reaction II, though less specific, would tend to provide additional information; thus, a *Positive* Reaction II, but *Negative* Reaction I, might strongly suggest the presence of a free amino group in a side chain at C-3. If this is the case, acetylation of this amino group should convert this compound into one in the *Slow*-reacting group under conditions of Reaction I.

The nature of the substituent on the pyrrole moiety was somewhat less important in determining the mode of action and the spectra of the colors formed. As expected, substituents with a positive inductive or mesomeric effect (CH_3, phenyl) increased the reactivity while those with negative inductive effects (COOH, CHO, $NH(CH_3)_2^+$) decreased the reactivity and often totally eliminated the reaction. Thus, within the *Slow*-reacting group under conditions for Reaction I, the substituent controlled the rate of the reaction to make skatole react fastest (half maximal color in 90 min) and indoleacetic acid slowest (remaining colorless for several days). At times ambiguity in classification might result from an activating substituent as in the case of 5-methyltryptamine.

These differences in reactivity of indole derivatives could also be utilized for quantitation of two or more derivatives in the same aliquot without the necessity of a prior separation. A quantitative method for the determination of two indole derivatives based on differences in reactivity between indole (Reaction I *Fast,* Reaction II *Negative*) and tryptophan (Reaction I *Negative,* Reaction II *Positive*) was developed (15).

SUMMARY

A colorimetric method for the partial characterization of indole derivatives based on their differences in reactivity with *p*-dimethylaminobenzaldehyde has been described. The possible applications and the limitations of this method are discussed.

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