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SHORT COMMUNICATION

AN IMPROVED PROCEDURE FOR THE SYNTHESIS OF OXINDOLE-3-CARBINOL (HYDROXYMETHYL OXINDOLE)

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Abstract-Oxindole-3-carbinol was synthesized by the photochemical oxidation of 3-indoleacetic acid. The reaction was performed in buffered aqueous solution at pH 4·6 in the presence of riboflavin and using fluorescent lamps as an energy source. The molar extinction coefficient of oxindole-3-carbinol in 95 % ethanol was determined to be 21,300.

OXINDOLE-3-carbinol (I) was required during a study of the chemical effects of peroxyacetyl nitrate on indole metabolism in tobacco (Hope and Ordin, in preparation). Tuli and Moyed[1] have demonstrated that the dehydration product of I, 3-methylene oxindole (II), acts as a growth regulator in etiolated pea stem segments. Since I was unstable and not commercially available, it was desirable to synthesize it at regular intervals. The photochemical oxidation of 3-indoleacetic acid (III) to I and II in presence of riboflavin provides a facile one step route but this procedure as described by Fukuyama and Moyed[2] gave variable yields in our hands. The initial pH of a solution of 50 mg III plus 1·0 mg riboflavin in 50 ml glass distilled water was 3·87. Preliminary experiments in our laboratory using reaction mixtures buffered at pH values of 3·6, 4·6 and 5·6 indicated that a buffered reaction mixture gave consistent results, the greatest yield of I being from the pH 4·6 reaction.

In a typical synthesis, 50 mg III plus 1·0 mg riboflavin were dissolved in 50 ml pH 4·6 0·05 M acetate buffer and placed in a 14·5 cm dia. petri dish for exposure to a Sylvania 20W cool white fluorescent light. The fluorescent tube was approximately 1·5 cm above the surface of the reaction mixture. The petri dish containing reaction mixture was placed on a large sheet of chromatography paper and the fluorescent tube was backed with chromatography paper to increase the light intensity (1022 lx) in the region of the reaction mixture. Aliquots of reaction mixture (20 μl) were removed at 30 min intervals, diluted with buffer (2-O ml) and the absorption spectra from 220 to 300 nm recorded on a recording spectrophotometer. The intensities of absorption peaks at 279 (III) and 249 (I) nm were measured and are presented in Fig. 1. After 240 min the concentration of I reached a maximum and the fluorescent light was turned off. The reaction mixture was filtered through diatomaceous earth, freeze-dried and resolved on Whatman 3 MM paper in the dark at 2" using 2-propanol–pH 4·4 acetate buffer (5: 95, v/v). When viewed under short wavelength u.v. light I was located by its strong quenching on paper chromatograms at R_f 0·73.

This procedure yields I and II in the ratio of 6·55 : 1. The yield of I measured after initial chromatography is 5·2 %. It appears that the pH of the reaction mixture is critical since

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[1] V. TULI and H. S. MOYED, *J. Biol. Chem.* **244**, 4916 (1969).
[2] T. T. FUKUYAMA and H. S. MOYED, *J. Biol. Chem.* **239**, 2392 *(1964).*

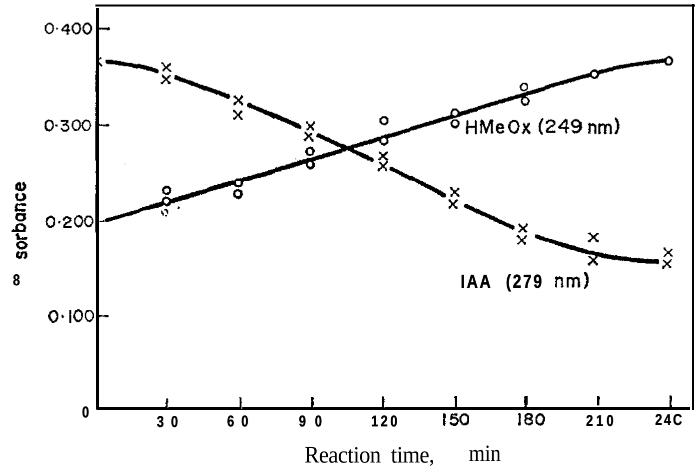

FIG. I. TIME-COURSE FOR PHOTOCHEMICAL OXIDATION OF 3-INDOLE-ACETIC ACID TO OXINDOLE-3-CARBINOL.

A reaction mixture containing 50 mg 3-indoleacetic acid and 1·0 mg riboflavin in 50 ml pH 4·6 0·05 M acetate buffer was exposed to fluorescent light for 240 min.

Fukuyama and Moyed[2] reported appreciable conversion of I to II in pH 5·0 buffered solution during a 12 hr period. Hinman and Lang[3] have proposed an indole epoxide (IV) illustrated in Fig. 2 as the immediate precursor of I during the peroxidase catalyzed synthesis of I from III. Both the proposed epoxide precursor of I and the fact that epoxide ring cleavage is favoured under acidic conditions are in agreement with our observation on pH dependence of the synthesis of I.

Following initial chromatography of the reaction mixture on 3 MM paper, the most successful purification procedure was elution of I with buffer or distilled water, freeze-drying and chromatography in n-butanol-benzene-methanol-water (20 : 30: 40 : 10, v/v). Concentration of I *in vacuo* caused rapid polymerization even at room temperature and was avoided.

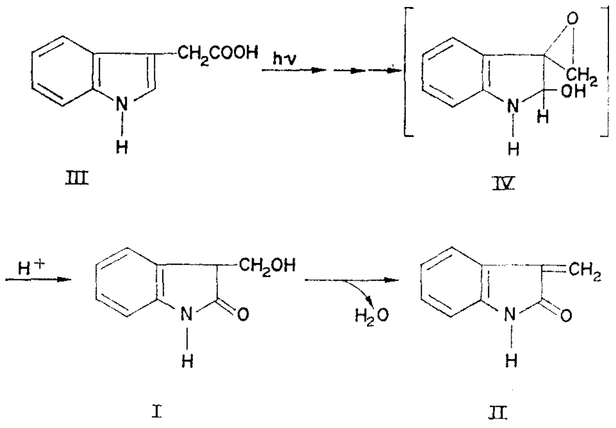

FIG. 2. PROPOSED PATHWAY OF FORMATION OF OXINDOLE-3-CARBINOL AND 3-METHYLENE OXINDOLE. Modified after Hinman and Lang.[3]

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[3] R. L. HINMAN and J. LANG, *Biochem, 4, 144 (1965).*

Indole chromogenic location reagents reacted slowly if at all with I. The spray reagent DMAC (1% p-dimethylaminocinnamaldehyde in **conc. HCl** diluted 1 vol. acidic DMAC to 10 vol. acetone) gave a faint yellow color turning pinkish red overnight. Ehrlich gave no detectable color reaction.

A comparative method for determination of a molar extinction coefficient for I was devised making use of the fact that I in alkali is rapidly converted to II. Because of its instability in solution at high **concentration**,[4] crystallization of I to high purity was not possible. Samples of I purified by paper chromatography were eluted with 95 % ethanol and adjusted in concentration to an absorbance of **0·5–0·7** at 249 nm. To the sample **0·02** ml 2 N **NaOH** was added, the sample allowed to remain at room temp. for 3 min then readjusted to **pH 4·0–4·5** with **1·11** N **HCl**. The absorption of II thus generated ***in situ*** was measured at 249 nm. From the published molar extinction coefficient of **II**[4] and the observed **absorbances** of I and II the molar extinction coefficient of I was calculated to be 21,300 (SD. of mean = 990).

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[4] R. L. **HINMAN** and C. P. BAUMAN, ***J. Org. Chem.*** **29, 2431** ***(1964).***