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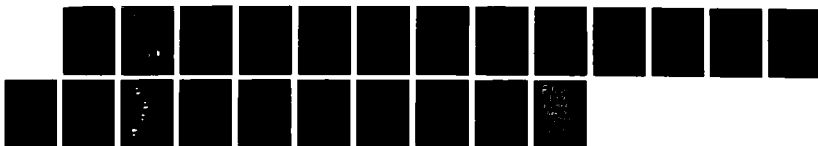
SYNTHESIS OF 1-METHYLINDOLE-3-ACETALDEHYDE AND ITS
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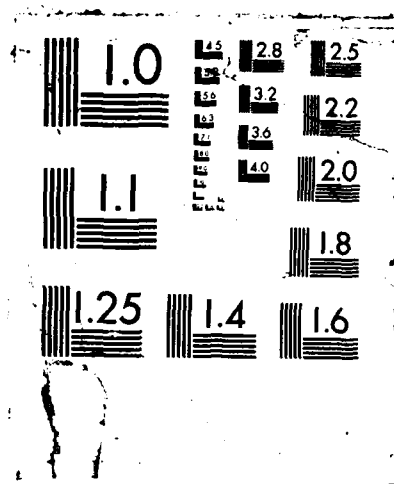
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SYNTHESIS OF 1-METHYLINDOLE-3-
ACETALDEHYDE AND ITS OXIDATION
BY HORSERADISH PEROXIDASE

by Homer R. Yeh, Ph.D.

RESEARCH DIRECTORATE

October 1987

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19 ABSTRACT (Continue on reverse if necessary and identify by block number) 1-Methylindole-3-acetaldehyde (1-methyl-IAAId) was synthesized in my laboratory from 1-methyltryptophan by interaction of the amino acid with dilute hypochlorite solution. 1-Methyl-IAAId was obtained in the form of bisulfite salt and the free aldehyde was a colorless syrupy liquid. It gave positive tests to Schiff and hydrazine reactions for aldehyde and to Salkowski, Ehrlich and xanthidrol reactions for indole. The I.R. spectrum of the freshly released 1-methyl-IADId bears a great similarity to that of indole-3-acetaldehyde (IAAId) except the NH stretching band at 3390 cm ⁻¹ is absent. 1-Methyl-IAAId shows strong to medium-strong bands at 1720, 1470, 1430, 1370, 1335, 1250, 1160, 1130, 1070, 1015 and 740/cm ⁻¹ and the typical indole absorption at 288 nm. The structure of the aldehyde was further confirmed by elementary analysis. Oxidation of 1-methyl-IAAId by horseradish peroxidase at acidic pH gave 1-methylindole-3-carboxaldehyde (1-methyl-IAAId) as the major product. The enzyme was not active in oxidation of 1-methyl-IAAId at pH above 6.0. At alkaline pH, bisulfite stimulated			
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19. ABSTRACT (Continued)

the catabolism of the aldehyde. However, unlike IAAld, peroxidation of 1-methyl-IAAld did not yield 1-methyl-4-hydroxyquinoline as a major product at pH above 6.0.

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PREFACE

The work described in this report was authorized under Project No. 1L161102A71A, Research in Chemical & Biological Defense, Biotechnology. This work was started in January and completed in February 1987. The experimental data are recorded in Laboratory Notebook 870028.

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SYNTHESIS OF 1-METHYLINDOLE-3-ACETALDEHYDE AND ITS OXIDATION BY HORSERADISH PEROXIDASE

1. INTRODUCTION

In an earlier study, we reported that indole-3-carboxaldehyde (IAld) was one of the major products in the peroxidase oxidation of indole-3-acetaldehyde (IAAld).¹ The catalytic reaction produced an aldehyde (IAld) with a side chain having one carbon shorter than the original aldehyde (IAAld). The optimal pH of the enzyme reaction was in the range of 4.4. At pH above 6.0, the enzyme showed little activity in oxidation of the aldehyde; whereas the presence of sodium bisulfite restored the enzymatic activity at alkaline pH.² One of the major products in the peroxidase-catalyzed oxidation of IAAld was found to be 4-hydroxyquinoline (4-HQ).¹

The possibility that 4-HQ can be produced as an oxidation product of indole-3-acetic acid (IAA) has been discussed by Manning and Galston.³ They believed that 4-HQ was not formed in the peroxidase oxidation of IAA. However, our work definitely shows that 4-HQ can be generated from either IAA or IAAld in the peroxidase-catalyzed reaction at alkaline pH in the presence of sodium bisulfite.^{1,4} At the optimal pH of the enzyme, 3-methylene oxindole has been identified to be the major product in peroxidase oxidation of IAA.⁵ No doubt, the reaction conditions have played a significant role in controlling the distribution of the oxidation products in the catalytic breakdown of these indole derivatives.

To illustrate the possible mechanism of the oxidation reactions, 1-methyl-indole-3-acetaldehyde (1-methyl-IAAld) was synthesized in my laboratory and the catalytic oxidation by the peroxidase was investigated. The results that we obtained indicated that, similar to what was observed for IAAld, the oxidation of 1-methyl-IAld yielded an aldehyde (1-methyl-IAld) having a side chain one carbon shorter than the original aldehyde. At alkaline pH, bisulfite also stimulated the oxidation of the aldehyde by the enzyme. However, the corresponding 1-methyl-4-HQ was not identified as a product in the oxidation reaction. Possible mechanisms of the peroxidase-catalyzed oxidation of these indole derivatives at different reaction conditions will be discussed.

2. MATERIALS AND METHODS

1-Methylindole-3-acetaldehyde (1-methyl-IAAld) was synthesized from 1-methyltryptophan according to a procedure described by Gray.⁶ 1-Methyltryptophan was, in turn, prepared by interaction of tryptophan with methiodide in liquid ammonia in the presence of metallic sodium according to a procedure outlined by Yamada et al.⁷ 1-Methyl-4-HQ was prepared from 4-HQ by a method reported by Simpson and Wright.⁸ 1-Methyl-IAld was synthesized from 1-methylindole by reaction of dimethylformamide on the indole in the presence of phosphorus oxychloride by a method described by Tyson and Shaw.⁹ Crystalline horseradish peroxidase (RZ=3.0) was purchased from Worthington Biochemical Corp., Freehold, N.J. All other chemicals and reagents were reagent grade or the highest purity and were used without further purification.

The rate of the enzymatic reaction was monitored by the spectral changes at 248 nm, which represented the overall reaction of the peroxidase oxidation of 1-methyl-IAAld. Infrared spectra were obtained in a Beckman infrared spectrophotometer. Thin-layer chromatography was run on either Brinkman silica gel or Eastman silica sheets with or without fluorescent indicator.

3. RESULTS

3.1 Synthesis of 1-Methylindole-3-Acetaldehyde.

The compound was synthesized according to a procedure described for IAAld by Gray⁶ and was also obtained in the form of bisulfite salt. It was recrystallized by dissolving the crude product in a minimum amount of water and then diluting with isopropanol to a final concentration of 90% isopropanol. Yield: 1.5 gm of 1-methyl-IAAld-NaHSO₃ from 4 gm of 1-methyl-tryptophan.

1-Methyl-IAAld was a colorless syrupy liquid. It gave positive tests to Schiff's and 2,4-dinitrophenylhydrazine reactions for aldehydes, and to Ehrlich and xanthydrol reactions for indole ring.

Figure 1 shows the infrared spectra of IAAld-NaHSO₃ (A), 1-methyl-IAAld-NaHSO₃ (B), IAAld (C), and 1-methyl-IAAld (D). As may be seen, the infrared spectrum of the freshly released 1-methyl-IAAld bears a great similarity to that of the free IAAld except that the N-H stretching band is not shown at 3390 cm⁻¹. The infrared spectrum of 1-methyl-IAAld-NaHSO₃ resembles that of IAAld-NaHSO₃. The carbonyl stretching band at 1720 cm⁻¹ of the free 1-methyl-IAAld (Figure 1D) cannot be seen in 1-methyl-IAAld-NaHSO₃.

The UV absorption spectrum of the free 1-methyl-IAAld had a maximum at 288 nm (molar extinction coefficient 5.2×10^4) and a trough at 247 nm.

Analysis of C₁₁H₁₂O₄NSNa:

Calcd. C, 47.65; H, 4.36; N, 5.05%

Found. C, 46.04, H, 4.86; N, 4.93%

3.2 Effect of pH on the Oxidation of 1-Methyl-IAAld and 1-Methyl-IAAld-NaHSO₃.

The effect of pH on peroxidase oxidation of these substrates is shown in Figure 2. The absorbance change at 247 nm (the trough) was used to monitor the overall rate of the enzymatic reaction. The reactions are seen to be pH dependent; in the pH range of 3.5 to 4.4, a known optimal pH for peroxidase oxidations, the two substrates were rapidly oxidized although the presence of equimolar NaHSO₃ slightly inhibited the reaction of the free aldehyde. In the pH range of 4.4 to 6.2, the reaction rates decreased with increasing pH. Above pH 6.2, a distinct difference in the rate of oxidation can be seen; as the pH was increased, the rate of oxidation of the free aldehyde decreased; whereas the oxidation of the bisulfite salt increased as pH increased. Similar results were also reported for IAAld,² indicating a significant effect of NaHSO₃ on the peroxidase-catalyzed oxidation of these indole derivatives.

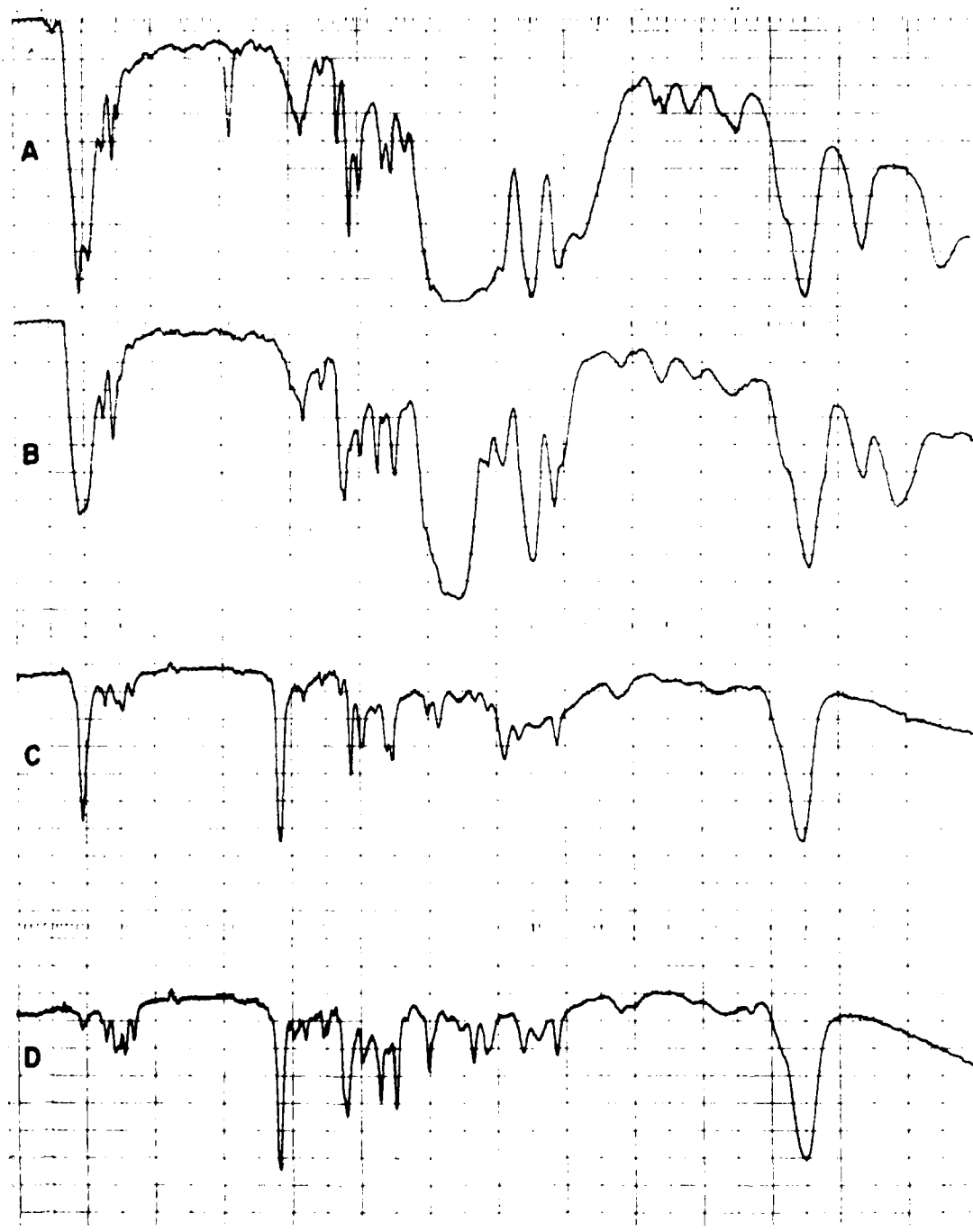


Figure 1. Infrared Absorption Spectra of Indole-3-Acetaldehyde Sodium Bisulfite Salt (A), 1-Methylindole-3-Acetaldehyde Sodium Bisulfite Salt (B), Indole-3-Acetaldehyde (C), and 1-Methylindole-3-Acetaldehyde (D)

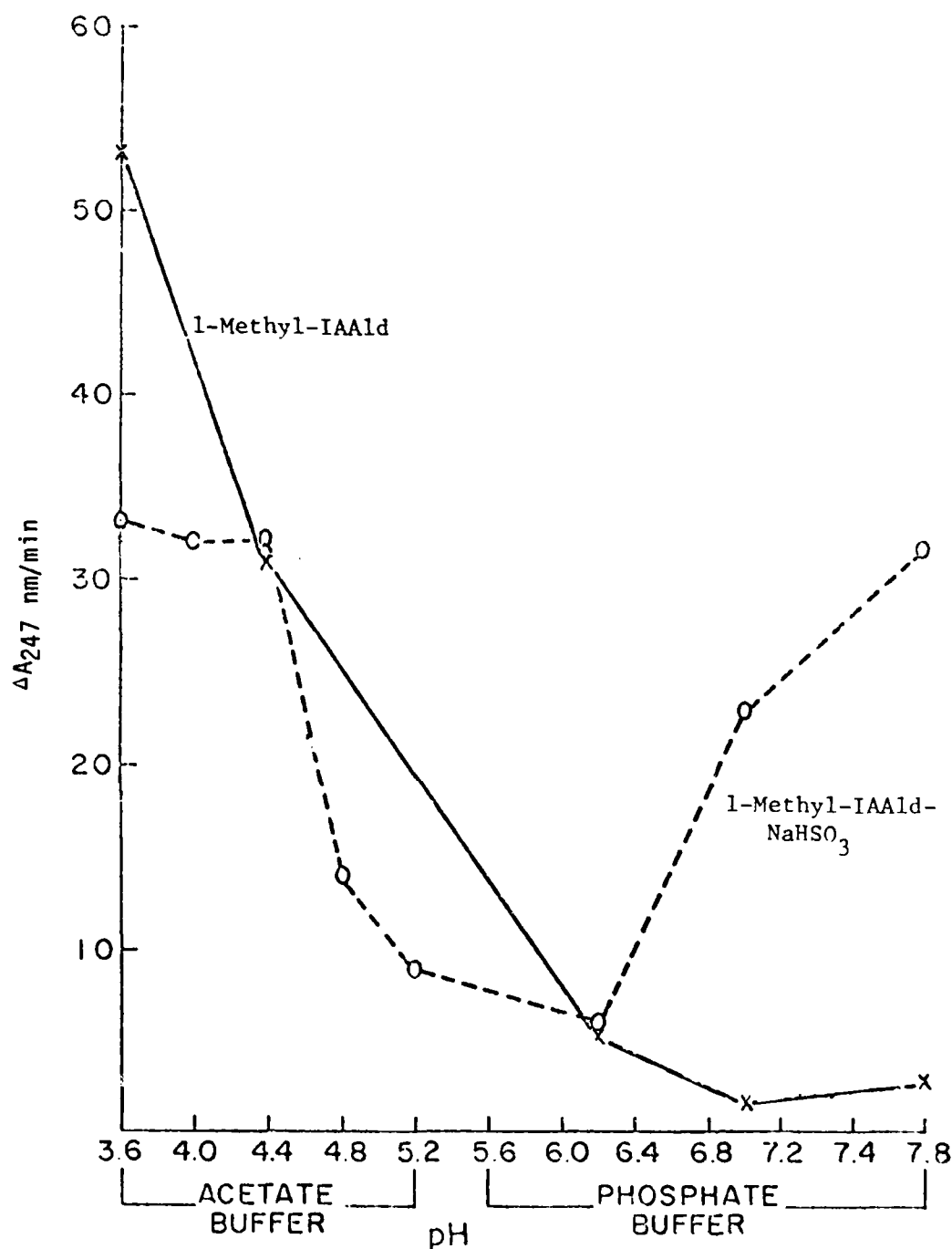


Figure 2. Effect of pH on the Oxidation of IAA, IAAld, IAAld-NaHSO₃

The rate of the enzymatic reaction was followed by the change of absorbance at 247 nm per hour. Reaction mixture contained 1×10^{-4} M of substrates, IAA, IAAld, or IAAld-NaHSO₃ in 0.05 M of buffer solution, 6 μ g of enzyme (RZ = 2.0), and 1×10^{-4} M of H_2O_2 . For pH values ranging from 3.6 to 5.2 and from 5.6 to 7.8, acetate and phosphate were used, respectively.

3.3 Spectrophotometric Studies on the Oxidation of 1-Methyl-IAAld.

The changes in ultraviolet spectra during the course of the peroxidase oxidation of 1-methyl-IAAld at pH 5.0 are shown in Figure 3. Oxidation of the aldehyde caused immediate changes in the spectra at 247- to 249-, 265-, and 303-nm regions. At equilibrium, the spectrum showed no similarity to that of 3-methylene indole and that of 1-methyl-4-HQ. The formation of 1-methyl-IAld could not be shown by spectrophotometric method since 1-methyl-IAld and 1-methyl-IAAld have very similar ultraviolet absorption spectra. 1-Methyl-IAld has a single maximum at 288 nm with a trough at 261 nm and 1-methyl-IAAld shows a maximum at 292 nm and a trough at 247 nm. At alkaline pH, the changes in the spectrum during the course of oxidation of 1-methyl-IAAld-NaHSO₃ showed no significant difference from those observed for free aldehyde at acidic pH.

3.4 Thin-Layer Chromatographic Evidence for the Formation of 1-Methyl-IAld from Oxidation of 1-Methyl-IAAld.

Although the formation of 1-methyl-IAld cannot be demonstrated by spectrophotometric method, subsequent thin-layer chromatographic analysis indicated that 1-methyl-IAld was formed as a major product in the enzyme oxidation of 1-methyl-IAAld. The results of thin-layer chromatography of the oxidation product of 1-methyl-IAAld in four solvent systems are shown in Figure 4, and the R_f values are summarized in Table 1 for comparison. The R_f values of the oxidation product corresponded well with the authentic 1-methyl-IAld. The spots were identified by spraying the thin-layer plates with 2,4-dinitrophenyl-hydrazine reagent. Furthermore, when the reaction was performed at alkaline pH in the presence of NaHSO₃, the formation of 1-methyl-4-HQ was not demonstrated by either thin-layer chromatographic or spectrophotometric method. Apparently, product or products other than 1-methyl-4-HQ were produced as a result of the oxidative degradation of 1-methyl-IAAld. Additional work is required to identify the structural characteristics of the unknown product(s).

4. DISCUSSION

At the optimal pH of the enzyme reaction, the oxidative breakdown of IAA, IAAld, and 1-methyl-IAAld all resulted in the formation of the oxidation products that are one carbon less than the original compound, and the missing carbon atom is usually derived from the α -carbon of the side chain. It is likely that the enzymatic oxidation of these indole derivatives follows the same reaction mechanism that leads to the formation of different products. Evidence available to date suggests the transient existence of a 3-methylene indolenine-like intermediate in the enzyme-catalyzed oxidation of these indole derivatives.¹⁰ The α -carbon oxidative elimination process may involve the protonation of the electronically dense C-3 position of the indole ring.¹¹ The site of oxidation is then determined by the nature of the α -carbon and the presence or absence of an additional group at the N-1 or C-2 position.

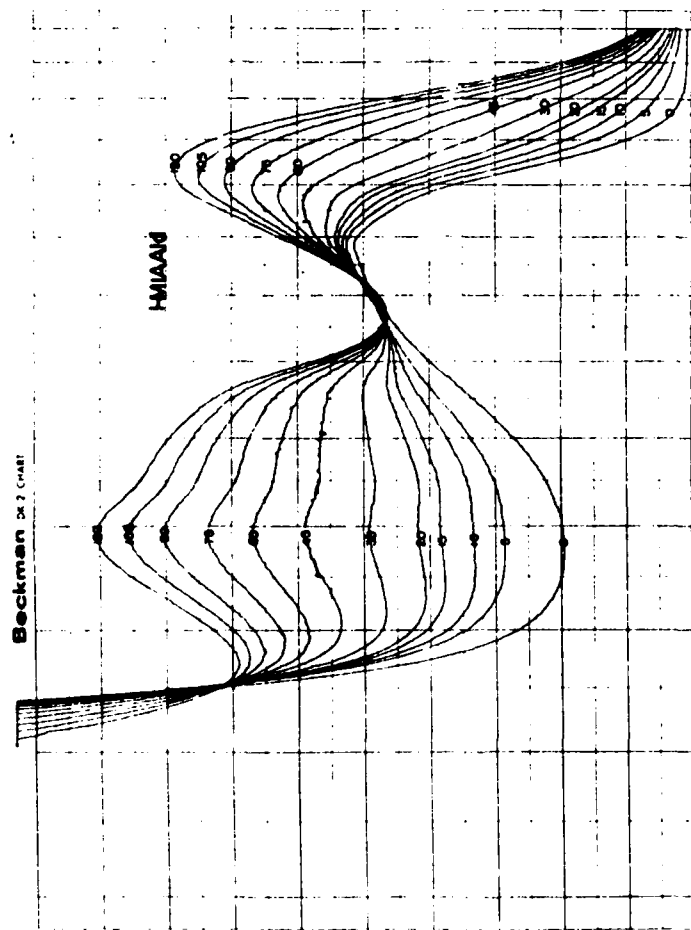


Figure 3. Changes in Ultraviolet Absorption Spectrum During Enzymatic Oxidation of 1-Methylindole-3-Acetaldehyde (1-Methyl-IAAId)

0.1 ml of enzyme (17 μ g per cuvette of 3.2 ml), 3 ml of 1.07×10^{-4} M 1-methyl-IAAId in 0.05 M of acetate buffer solution, pH 4.85, and 0.1 ml of 1×10^{-2} M of H_2O_2 solution. Total volume of the reaction mixture was 3.2 ml per cuvette. Records were begun at 340 nm. Curves were taken at 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 minutes; each record was completed in 1 minute. Final concentration of 1-methyl-IAAId was 1×10^{-4} M and that of H_2O_2 was 3×10^{-4} M.

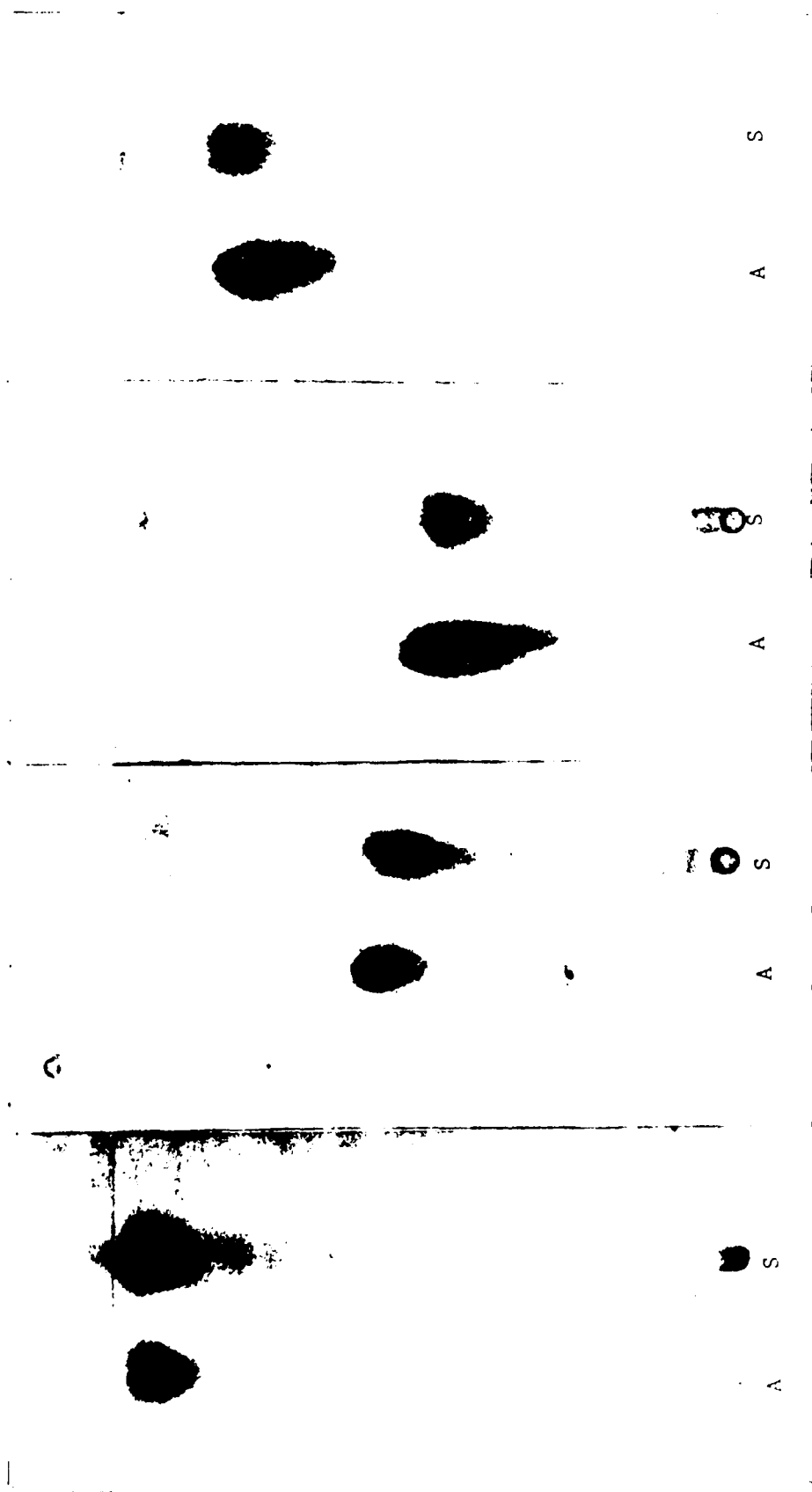


Figure 4. Thin-Layer Chromatogram of 1-Methylindole-3-Carboxaldehyde Obtained by Oxidation of 1-Methylindole-3-Acetaldehyde

Plates were Eastman Chromatogram Sheet (Type K301, R2 silica gel without fluorescent indicator). Solvent systems were (a) n-propanol-hexane (1:4), (b) chloroform-acetic acid (95:5), (c) benzene-acetic acid-water(4:1:1), and (d) n-butanol-hexane (18% n-butanol). Authentic spots of 1-methylindole-3-carboxaldehyde were marked as "A," and the spots from oxidation of 1-methylindole-3-acetaldehyde were marked as "S." The spraying reagent was 2,4-dinitrophenylhydrazine in ethanol.

Table 1. R_f Values of 1-Methyl-IAld and Isolated 1-Methyl-IAld

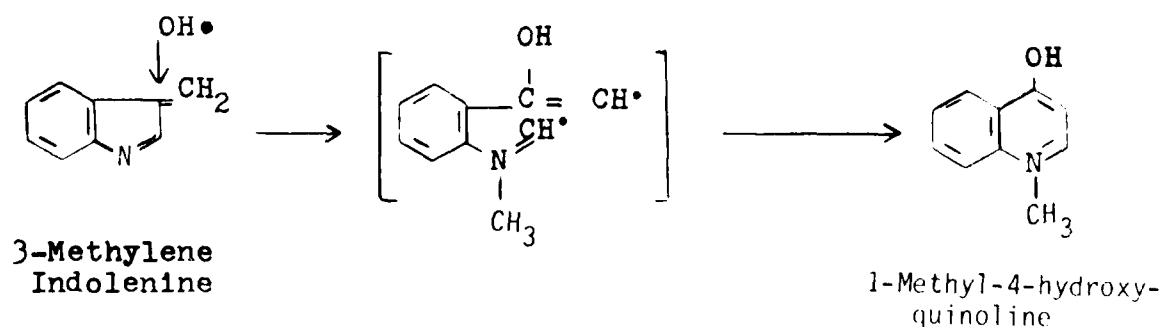
Thin-Layer Plates	Solvent System	Isolated	
		1-Methyl-IAld	1-Methyl-1-Methyl-IAld
Eastman Chromatogram Sheet	n-Propanol-Hexane	0.94	0.94
	(1:4)		
	Chloroform-Hexane	0.56	0.54
	(95:5)		
	Benzene-Acetic Acid-Water	0.46	0.45
Silica Gel F ₂₅₄	(4:1:1)		
	n-Butanol-Hexane	0.78	0.80
	(18% n-Butanol)		
	n-Butanol-Hexane	0.30	0.83
	(18% n-Butanol)		

1-Methyl-4-HQ is a naturally occurring alkaloid that was first isolated from *Echipses* by Greshoff¹² in 1933 and was called echipsin. Since 1-methyl-4-HQ was not identified in the peroxidase oxidation of 1-methyl-IAAld, echipsin may be produced directly from methylation of 4-HQ rather than from 1-methyl-IAAld.

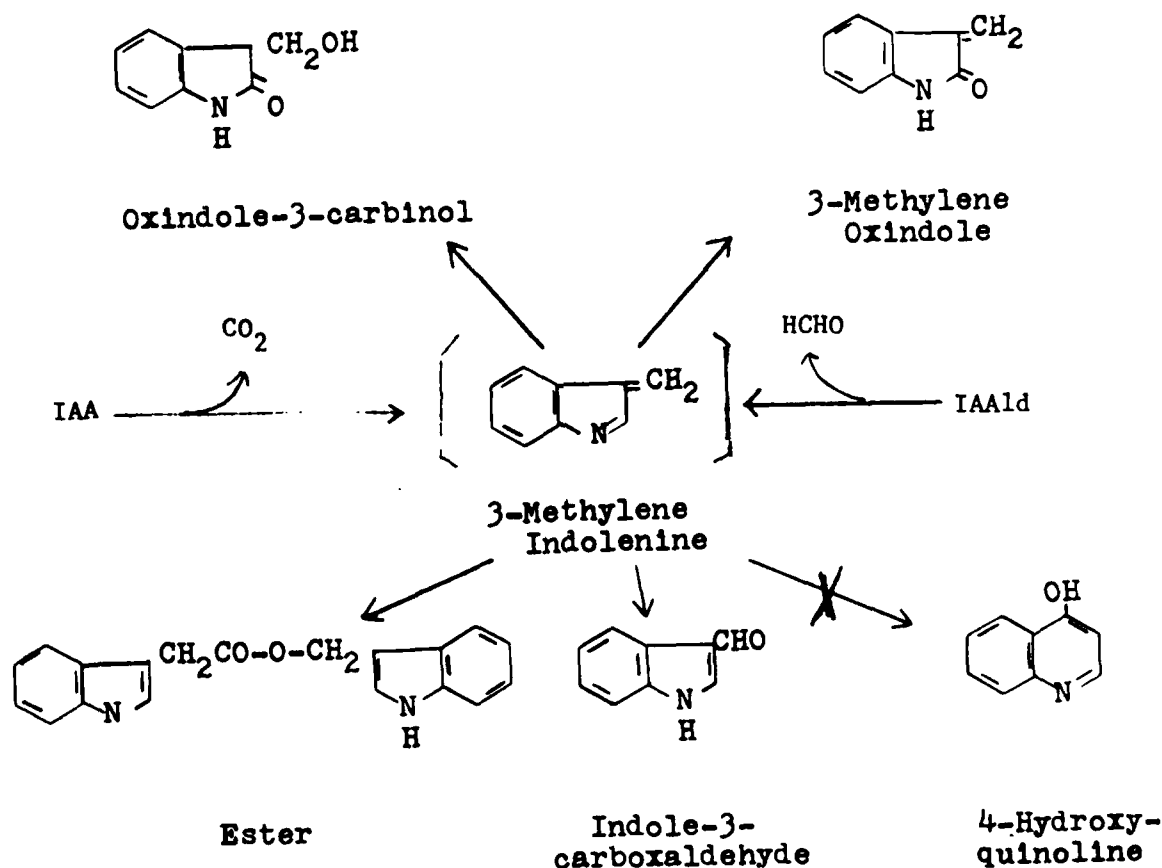
At alkaline pH, the enzyme usually is inactive toward the oxidation of these indole derivatives. The stimulatory effect of NaHSO_3 on the peroxidase oxidation of these compounds may involve the generation of free radicals that are essential for the oxidation of these indole derivatives at alkaline pH. Sodium bisulfite has been used as a sensitive detector for the generation of free radicals by Alyea and Backstrom¹³ and also by Fridovich and Handler.¹⁴ The mechanism involved in the bisulfite-stimulated oxidation of these indole derivatives may be similar to the bioluminescence generated by xanthine oxidase in the presence of luminol or lucigenin, which depend on the generation of free radicals.¹⁵

We have reported that 4-HQ was produced in the NaHSO_3 -stimulated peroxidase oxidation of IAAld.² The formation of 1-methyl-4-HQ from 1-methyl-IAAld, however, was not demonstrated. The different results indicate a significant effect of the 1-methyl group in the ring expansion reaction. The question is raised as to whether the 3-methylene indolenine-like intermediate may also be involved in the formation of 4-HQ from IAAld. If this mechanism is involved in the catalytic formation of 4-HQ from IAAld, the attachment of a methyl group at the N-1 position would not hamper the formation of 1-methyl-4HQ from 1-methyl-IAAld. This concept is depicted in the following scheme.

At alkaline pH:

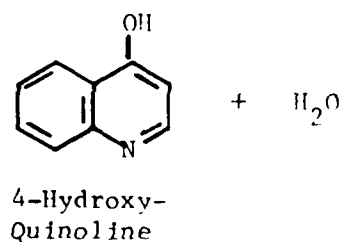
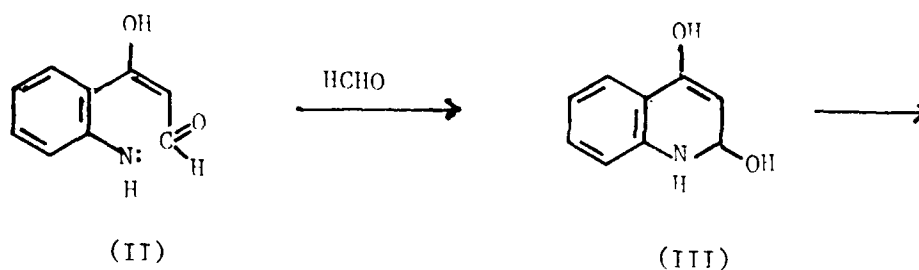
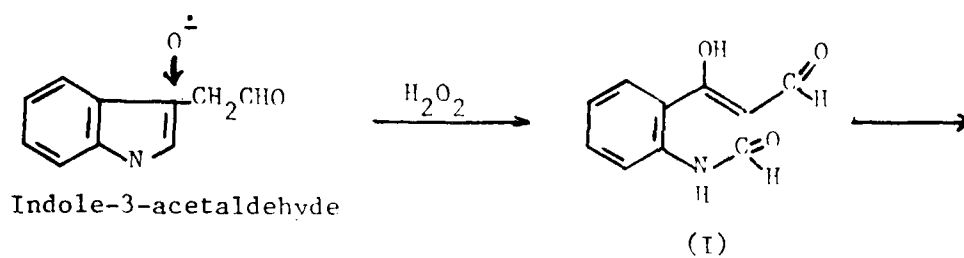


At acidic pH:

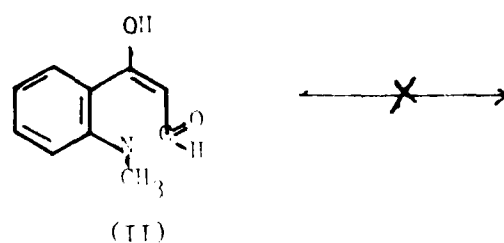
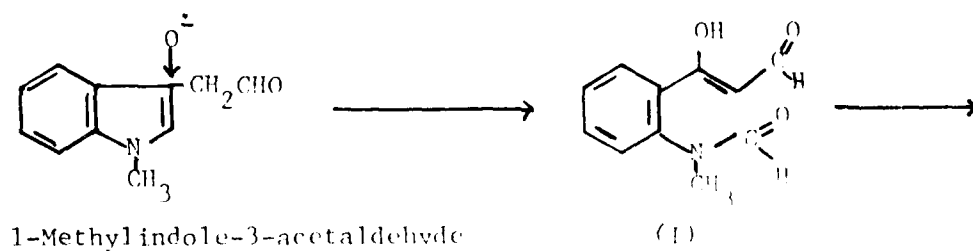


Since 1-methyl-4-HQ was not generated as a product, the oxidation of the indole-3-acetaldehyde derivatives may follow a reaction mechanism distinct from that observed at acidic pH. The sequence of the reaction may be similar to that of the well-known kynurenine pathway for tryptophan metabolism, which involves the cleavage of the indole ring at the 2,3-position and results in the loss of the 2-carbon atom of the indole ring instead of the α -carbon of the side chains. This reaction mechanism requires an additional equivalence of H_2O_2 essential for generation of the free radicals. The mechanism may be depicted in the following schemes:

4-Hydroxyquinoline:



1-Methyl-4-HQ: The presence of the 1-methyl group may hamper the closure of the ring and 1-methyl-4-HQ was not produced.



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