The Conversion of Indole-3-Acetic Acid to 3-Methylene-oxindole in the Presence of Peroxidase.

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The existence of enzyme systems in higher plants that catalyze the oxidative degradation of the plant growth hormone indole-3-acetic acid (IAA) has been recognized for many years and numerous unsuccessful attempts have been made to identify the organic products of the oxidation (Ray, 1958). Recognizing that the IAA-oxidase systems are of the peroxidase type (Ray, 1958), and that the natural oxidases and purified peroxidases would probably yield closely related if not identical products, we report here the first conclusive proof of structure of the principal product from the oxidation of IAA, catalyzed by crystalline horseradish peroxidase, in the absence of added hydrogen peroxide.

Experimental. 3-Bromooxindole-3-acetic acid, prepared by the reaction of N-bromosuccinimide and IAA by a procedure to be described in a forthcoming paper, melted at 150-151° dec. Anal. Calcd. for $C_{10}H_8BrNO_3$; C, 44.46; H, 2.98; N, 5.19; Br, 29.59. Found: C, 44.59; H, 3.20; N, 5.05; Br, 29.80.

Enzymatic reactions were carried out as described previously (Hinman and Frost, 1961). Final concentrations were 10^{-4} M IAA and 10^{-7} M peroxidase (calculated by assuming that the weighed protein was pure enzyme of M.W. 44000). IAA, measured by the modified Salkowski test (Gordon and Weber, 1951), was completely consumed in about one hour. Formation of the final product, observed spectrophotometrically, continued for upwards of

48 hours at 25°, but was complete in 10-15 minutes at 100°. Similar spectral changes were observed at pH 3.5 (0.1 M citrate), pH 5.0 (0.2 M acetate), and pH 6.5 (0.067 M phosphate).

Reactions with sodium bisulfite were effected by adding 0.05 - 0.10 ml. of 5% aqueous sodium bisulfite to 2.5 ml. of reaction mixture at pH 5.0.

All spectra were measured in 0.2 M acetate buffer at pH 5.0. Those shown in Fig. 1 are: (1) IAA (10^{-4} M); (2) IAA (10^{-4} M) + peroxidase (10^{-7} M) after 26 hrs. at 25°, or after 77 min. at 25° followed by 11 min. at 100°; (3) No. 2 + $NaHSO_3$; (4) Water layer of No. 3 after extraction with ether and correction for blank; (5) Dec. product of 3-bromooxindole-3-acetic acid

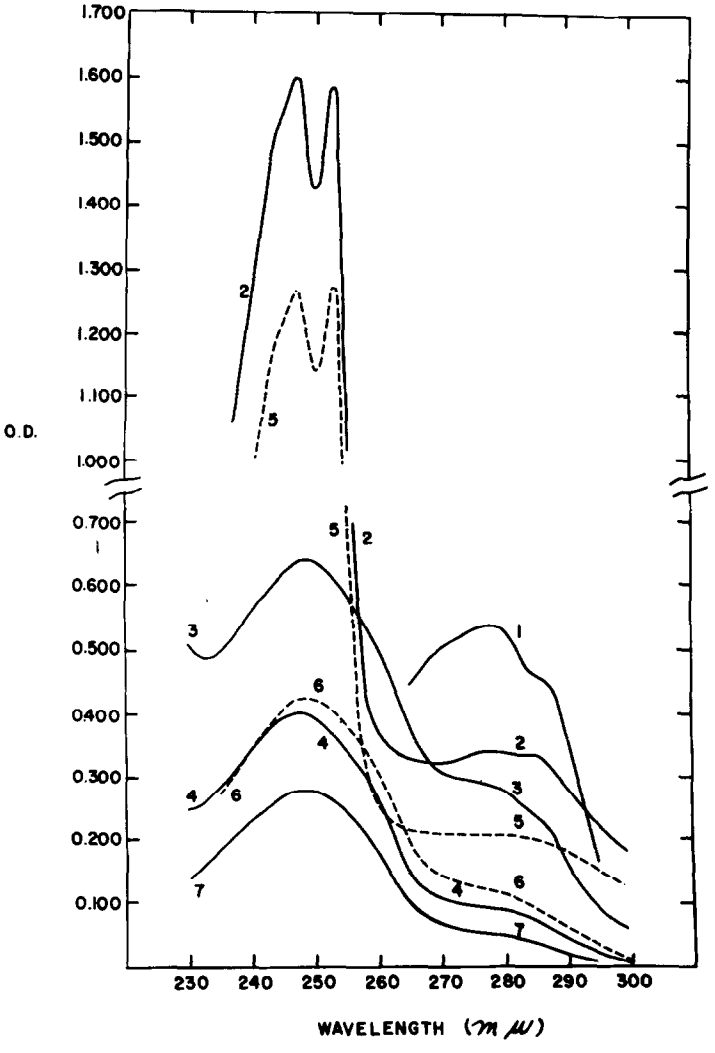


Fig. 1

(5×10^{-5} M); (6) No. 5 + $NaHSO_3$; (7) 3-Methyloxindole (10^{-4} M) (ordinate x 1/3).

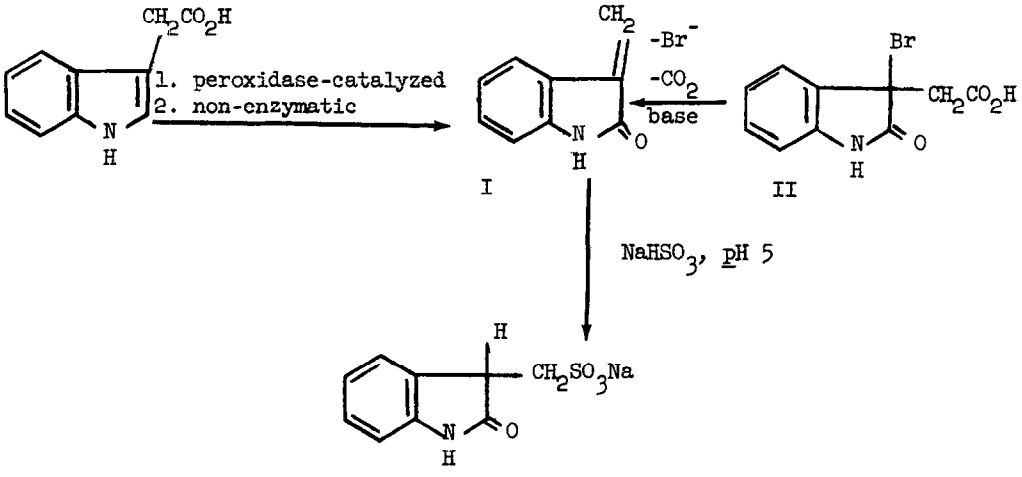
Discussion. We have found that the peroxidase-catalyzed oxidation of IAA is concentration-dependent. From 10^{-5} - 10^{-4} M IAA the oxidation is composed of two stages, the first of which is enzyme-catalyzed (Ray, 1956). In this concentration range the principal product of the overall reaction is 3-methyleneoxindole (I). A non-acidic indolic component is also formed. The proportion of the second product increases with increasing IAA concentration; at 10^{-3} M and above only indolic material is formed. The structure of this product is under investigation.

Structure I was suggested by the two maxima near 250 mμ in its ultraviolet spectrum (Fig. 1), a feature characteristic of oxindoles bearing an exocyclic double bond at the 3-position (Wenkert, Bernstein, and Udelhofen, 1958; Julian and Printy, 1953). An ultraviolet spectrum with peaks at the same wavelengths was obtained when 3-bromooxindole-3-acetic acid (II) was dissolved in water.

The conversion of II to I is base-catalyzed and bromide ion is released together with one mole of carbon dioxide per mole of II, indicating a combined dehydrohalogenation and decarboxylation, in a manner analagous to the base-catalyzed conversion of β-bromo-β-phenylpropionic acid to styrene (Fittig and Binder, 1879). The spectral features of I (λ_{max} 247, 253, 278 sh; ϵ_{max} 25,400; 25,500; 4200), as prepared from II, show the expected relationship to those of the known 3-ethylideneoxindole (λ_{max} 244 sh, 247, 253, 287; ϵ_{max} 25,200; 25,700; 29,600; 4550) and 3-isopropylideneoxindole (λ_{max} 252, 258, 292; ϵ_{max} 26,100; 28,800; 6750).

As an α,β-unsaturated amide I would be expected to undergo facile addition to the exocyclic double bond. Addition of sodium bisulfite to either the oxidation product of IAA or the decomposition product of II produced a new product, the spectrum of which (λ_{max} 248.5, 275 sh; ϵ_{max} 8600; 2500 for the product derived from II) resembled closely that of the known 3-methyloxindole (λ_{max} 247.5, 275 sh; ϵ_{max} 8360; 1680) as would

be expected from the proposed structure: the sodium salt of 3-(sulfomethyl)-oxindole.



The additional product(s) formed in the enzymatic reaction, detected by maxima at 280 and 285 mμ in the spectra of both I and its reduction product (Fig. 1, curves 2 and 3), was removed from the bisulfite reaction mixture by extraction with ether. The spectrum of the aqueous phase (Fig. 1, curve 4), then showed the oxindolic features more clearly. The fact that the oxindolic material remained in the aqueous phase is in agreement with the proposed sulfonic acid structure, since 3-methyloxindole is readily extracted into ether.

Using the ϵ_{max} of I, calculated by assuming the conversion of II to I is quantitative, the conversion of IAA to I was estimated as 60% after 22 hrs. at 25° when the rate of reaction was very slow.

Solutions of 3-methyleneoxindole gave negative tests with Salkowski reagent, Ehrlich's reagent, Ekman's reagent, and diazotized sulfanilic acid. Although I was fairly stable at the lower concentrations (10^{-5} - 10^{-4} M), above 10^{-3} M the intensity of its spectrum diminished rapidly with time, and at 10^{-2} M a precipitate (probably polymeric) formed. (Material of this type was probably the product isolated by Ray and Thimann (1956) from the reaction

of IAA with the oxidase from Omphalia). Under chromatographic conditions, particularly in the ammoniacal solvents generally used for chromatography of IAA and its oxidation products, I underwent rapid decomposition. This instability of I, which precludes its isolation, helps to explain the multiplicity of products reported by other workers.

The conversion of IAA to I appears to be a fairly general pathway for the oxidation of IAA. The reported ultraviolet spectra for the photolytic oxidation of IAA (Ray and Curry, 1958) indicate that I is a principal product when light of 302 and 313 mμ is used. Moreover, it is now clear that the principal product from the reaction of IAA with acidified hydrogen peroxide (Hinman and Frost, 1961) is compound I. α,α-Dimethylindole-3-acetic acid (III) is similarly converted to 3-isopropylideneoxindole and it can be predicted with confidence that the last compound is also formed in the non-enzymatic stage of the oxidation of III in the presence of the oxidase from Omphalia (Ray and Thimann, 1956).

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