Plant Physiol. (1972) 50, 499–502

Auxin Activity of 3-Menthyleneoxindole in Wheat

Received for publication March 2, 1972

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ABSTRACT

A product of the enzymatic oxidation of indole-3-acetic acid, 3-methyleneoxindole, is at least 50-fold more effective than indole-3-acetic acid in stimulating the growth of wheat (*Triticum vulgare*, red variety) coleoptiles. Ethylenediaminetetraacetic acid can antagonize the growth-stimulating properties of the parent compound, indole-3-acetic acid, presumably by chelating Mn^{2+}, which is required for the enzymatic oxidation of indole-3-acetic acid. The growth stimulating effect of 3-methyleneoxindole, a product of the blocked reaction, on the other hand, is still evident in the presence of ethylenediaminetetraacetic acid. In the presence of 2-mercaptoethanol, indole-3-acetic acid fails to stimulate the elongation of wheat coleoptiles. The property of binding to sulfhydryl compounds including 2-mercaptoethanol is unique to 3-methyleneoxindole among indole-3-acetic acid and its oxidation products. These findings suggest that 3-methyleneoxindole is an obligatory intermediate in indole-3-acetic acid induced elongation of wheat coleoptiles.

Earlier reports indicate that MeOx,[1] an oxidation product of IAA, is an obligatory intermediate in several auxin-mediated plant growth responses, including stimulation of the growth of pea and mung bean stem segments and promotion of protein synthesis in pea stems (6). Stimulation of growth and promotion of protein synthesis by IAA can be diminished by inhibiting the enzymatic oxidation of IAA by chlorogenic acid; MeOx, a product of this reaction can effectively bypass the block. Furthermore, in the presence of reduced compounds such as glutathione, IAA is incapable of promoting the growth of stem sections. Since IAA cannot form adducts with sulfhydryl compounds, this finding suggests that IAA is first oxidized to MeOx, a sulfhydryl reagent, before any growth can occur. It is the purpose of the experiments described in this paper to determine whether MeOx has a similar role in monocots such as wheat.

MATERIALS AND METHODS

Chemicals. 3-Bromooxindole-3-acetic acid was prepared by reaction of IAA with N-bromosuccinimide (3). This compound on solution in water is rapidly converted to MeOx, which was purified by chromatography in isopropanol-water (5:95). Alternately, MeOx was obtained by allowing a solution of HMO to undergo complete dehydration to MeOx at 23 C for 18 hr. HMO was prepared by photooxidation of IAA in the presence of riboflavin (1).

Analytical Procedures. To determine the concentration of HMO, a sample was allowed to undergo complete dehydration to MeOx during storage overnight at room temperature. The concentration of MeOx and, therefore, that of the original HMO was calculated from absorption at 248 nm using the published extinction coefficient (2).

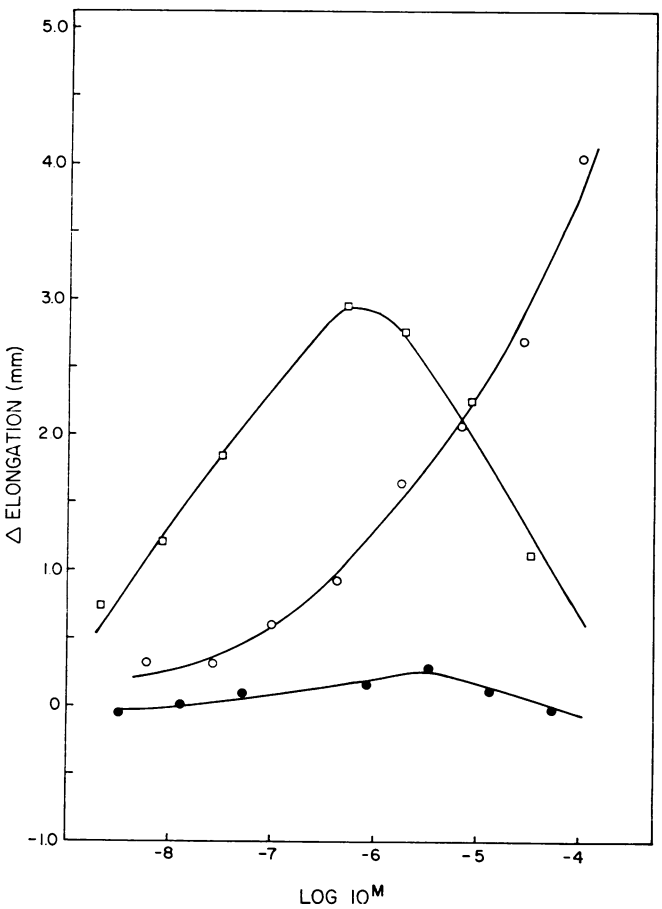

FIG. 1. Effect of IAA (○), and MeOx before (●) and after (□) chromatography of 3-bromooxindole-3-acetic acid on elongation of wheat coleoptiles. The experimental procedure is described in the text. Values of △ elongation shown were obtained after substracting the elongation obtained of wheat coleoptiles, grown in buffer alone.

Straight Growth Measurements. Wheat seeds (*Triticum vulgare*, red variety) were rinsed and soaked in distilled water for 16 hr at room temperature. The seeds were covered with moistened vermiculite and incubated at 23 C for 3 days in

[1] Abbreviations: MeOx: 3-methyleneoxindole; HMO: 3-hydroxymethyloxindole.

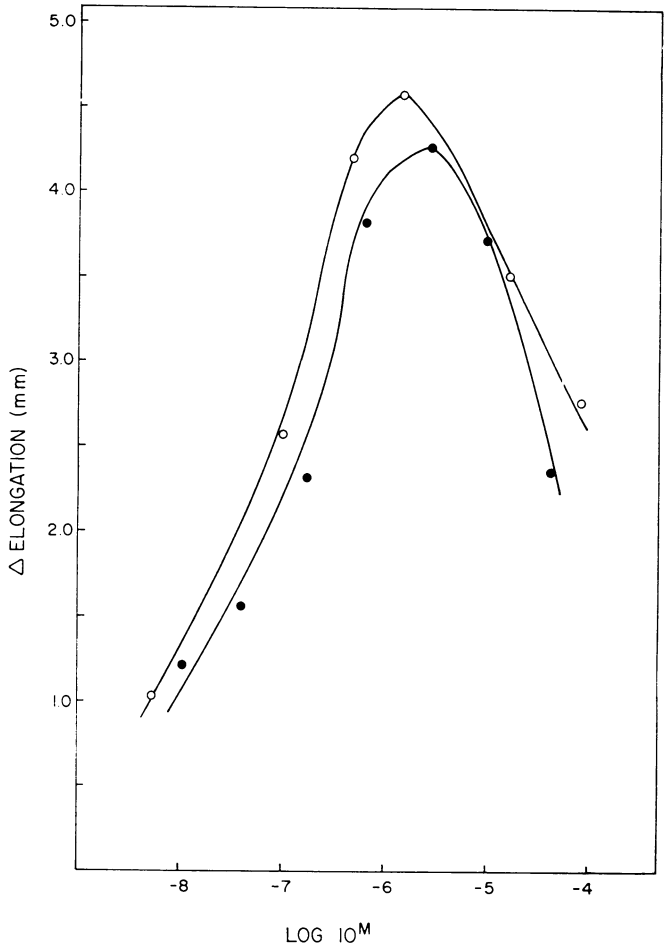

FIG. 2. Stimulation of growth of wheat coleoptiles by HMO (●) and MeOx (○). The experimental procedure and the method of preparation of MeOx from HMO are described in the text. Other conditions were similar to Figure 1.

total darkness. Coleoptile segments, about 7 mm in length, were cut just below the apical cap. For each determination five to eight such segments were partially submerged in 5 ml of 10 mM potassium phosphate buffer, pH 7.0, with the indicated additions contained in small Petri dishes. The segments were incubated at 23 C for 16 hr in total darkness. The length of the coleoptile segments was measured with a dissecting microscope fitted with a calibrated ocular micrometer. The segments which had undergone considerable curvature were held in a straightened position with forceps for measurement.

RESULTS AND DISCUSSION

Stimulation of Growth by IAA and MeOx. IAA can effectively stimulate the growth of wheat coleoptiles where growth appears to be almost a linear function of the concentration of IAA employed (Fig. 1). When prepared from 3-bromooxindole-3-acetic acid without further purification, MeOx does not stimulate growth. On solution in water, 3-bromooxindole-3-acetic acid yields in addition to MeOx, equimolar amounts of HBr and CO_2. The observed inhibition of growth, we felt, could be attributed to Br^-, especially since its toxicity in biological systems is well documented. Consequently, an aqueous solution of 3-bromooxindole-3-acetic acid was chromatographed in 5% isopropanol to obtain MeOx free of HBr. MeOx prepared in this manner was found capable of effectively stimulating the growth of wheat coleoptiles. The inhibitory effects of Br^- will be further substantiated in a subsequent section, where it will be demonstrated that IAA and its oxidation product, MeOx, fail to promote the growth of wheat coleoptiles in the presence of Br^-.

Stimulation of Growth by HMO and Its Dehydration Product. The observation that MeOx can effectively stimulate the growth of wheat coleoptiles in the absence of Br^-, prompted an investigation of the auxin activity of HMO, which eventually is dehydrated to MeOx and does not require Br^- for its preparation (1). The results presented in Figure 2 indicate that HMO is about 100 times more effective than IAA in terms of the concentration required for maximal stimulation of growth. Part of the solution of HMO used in this experiment was incubated at 23 C for 18 hr. Ultraviolet absorption spectra revealed that under such conditions HMO had been completely dehydrated to MeOx. Examination of the growth-promoting properties of MeOx, obtained as the dehydration product of HMO, indicate that it is as effective as the latter in stimulating the growth of wheat coleoptiles (Fig. 2).

Effect of HBr on IAA and MeOx-induced Elongation. Inhibition of growth of wheat coleoptiles exposed to unchromatographed MeOx obtained from solution of 3-bromooxindole-3-acetic acid led to the speculation that Br^-, which is generated upon dissolving 3-bromooxindole-3-acetic acid in water, was responsible for the inhibitory effect rather than MeOx. Consequently, a preparation of Br^--free MeOx, obtained after chro-

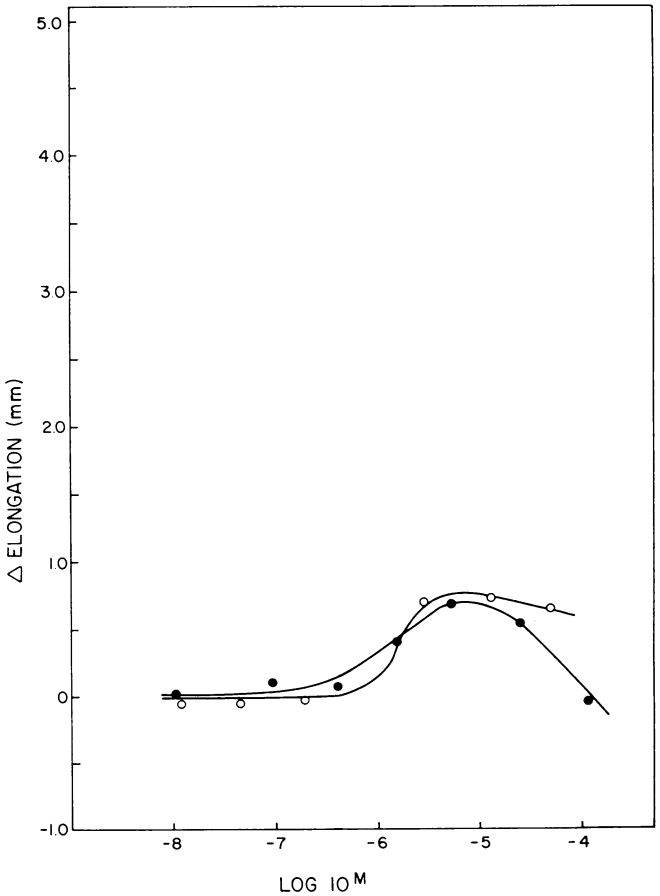

FIG. 3. Effect of bromine on IAA- and MeOx-stimulated elongation of wheat coleoptiles. HBr (0.5 mM) was used with varying concentrations of IAA (●) and MeOx (○), prepared as in Figure 2. Elongation obtained in presence of HBr was identical to that obtained in buffer; △ elongation values are expressed as in Figure 1.

matography of a 3-bromooxindole-3-acetic acid solution, was shown to be active in promoting the growth of wheat coleoptiles (Fig. 1). The inhibitory effect of Br^- is not specific for MeOx-induced growth, as Br^- administered in the form of 0.50 mM HBr is found to antagonize growth induced by IAA as well (Fig. 3). HBr is not a general poison, since the growth of wheat coleoptiles incubated in 0.50 mM HBr is not inhibited. The finding that growth induced by MeOx and its parent compound IAA is inhibited by HBr suggests that the stimulatory effects of IAA and MeOx may have a common biochemical origin.

Inhibition of Growth by EDTA. Any speculation ascribing an obligatory role for MeOx in auxin action has inherent in it the assumption that IAA is first oxidized to MeOx. Plants have an efficient means of accomplishing the oxidation via IAA oxidase, an enzyme which requires Mn^{2+} for optimal activity. Chelating agents such as EDTA would be expected to bind Mn^{2+}, thereby providing a rate-limiting step for the enzymatic oxidation of IAA. Such a system can provide a convenient means for testing the pertinence of MeOx in auxin action. If growth induced by IAA is indeed dependent upon prior oxidation to MeOx, then growth of wheat coleoptiles induced by IAA should be neutralized by EDTA as enzymatic oxidation of IAA to MeOx would be reduced. The growth-promoting properties of MeOx, however, must still be evident under such conditions, since it is a product of the blocked reaction. It may be seen in Figure 4 that growth resulting from application of IAA is greatly inhibited by EDTA, whereas the growth-promoting properties of MeOx are affected to a lesser extent by the chelating agent.

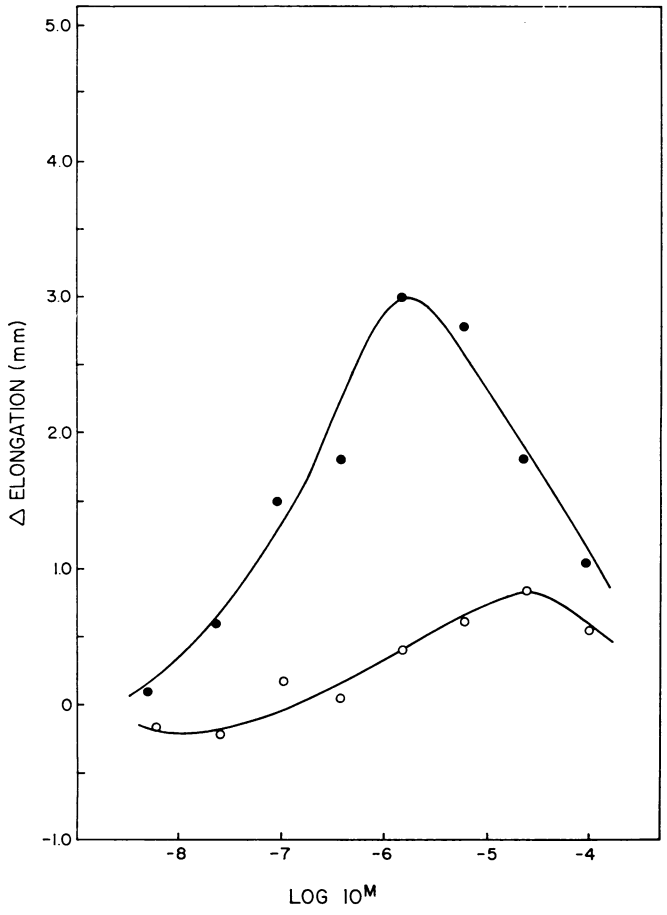

FIG. 4. Effect of EDTA on IAA- and MeOx-stimulated elongation of wheat coleoptiles. Varying concentrations of IAA (○) and MeOx (●), prepared as in Figure 2 with 1 mM EDTA. Experimental details and calculation of △ elongation are described in the text and in Figure 1, respectively. Elongation obtained in presence of EDTA was identical to that obtained in buffer alone.

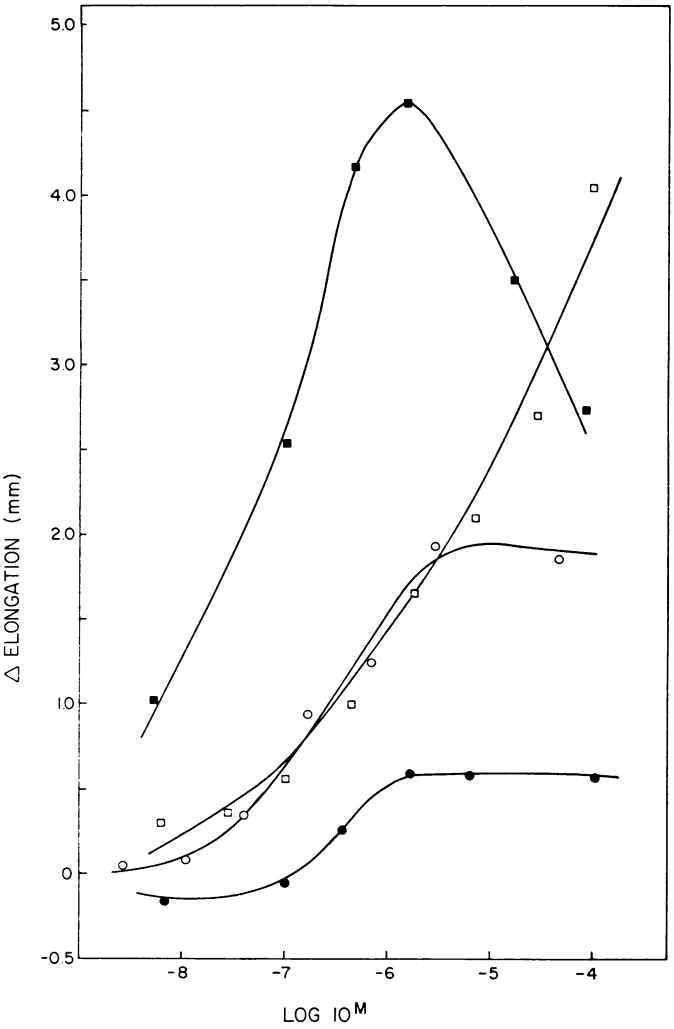

FIG. 5. Effect of 2-mercaptoethanol on IAA- and MeOx-induced growth in wheat coleoptiles. Growth of wheat coleoptiles in varying concentrations of IAA (□) and MeOx (■) obtained after dehydration of HMO. Growth of wheat coleoptiles in indicated concentrations of IAA (●) and MeOx (○) in the presence of 5 mM 2-mercaptoethanol. Other details are described in Figures 1 and 4.

Effect of 2-Mercaptoethanol on IAA- and MeOx-induced Growth. Failure of IAA to stimulate the growth of wheat coleoptiles in the presence of EDTA and the ability of MeOx to do so under identical conditions (Fig. 4) strongly suggest that MeOx is a key intermediate in auxin action. This conclusion is supported by an additional observation. As seen in Figure 5, stimulation of growth resulting from addition of IAA or MeOx is greatly inhibited by 2-mercaptoethanol. Unlike MeOx, IAA is not a sulfhydryl reagent, therefore, it would not form adducts with reduced compounds such as 2-mercaptoethanol and be rendered biologically inactive. However, the observation that IAA is unable to stimulate the growth of wheat coleoptiles in the presence of 2-mercaptoethanol indicates that stimulation of growth by IAA is mediated via prior oxidation to MeOx, a potent sulfhydryl reagent (4).

The results presented in this paper strongly suggest that

MeOx, an oxidation product of IAA, is a key intermediate in the stimulation of growth of wheat coleoptiles mediated by IAA. This conclusion is based on the following observations.

1. In the presence of chelating agents such as EDTA, IAA is incapable of stimulating the growth of wheat coleoptiles (Fig. 4). It is suggested that the chelating agent binds Mn^{2+}, a cofactor for the enzymatic oxidation of IAA, thereby interrupting a continuing supply of MeOx, a product of the blocked reaction. This hypothesis is supported by the finding that MeOx can effectively stimulate the growth of wheat coleoptiles in the presence of EDTA (Fig. 4).

2. Growth of wheat coleoptiles resulting from application of IAA can be abolished by 2-mercaptoethanol, a sulfhydryl compound (Fig. 5). A reasonable interpretation of this finding is that IAA is first metabolized to MeOx, a sulfhydryl reagent, before any growth occurs.

We feel that metabolism of IAA via the oxindole pathway (5) generates MeOx, the key intermediate in some auxin-linked phenomena.

Acknowledgments—The authors are grateful to Dr. H. S. Moyed for his advice and encouragement throughout the course of these investigations. This research was supported by grants from the National Science Foundation (GB-15898) and the National Institutes of Health (GM-17521) awarded to Dr. H. S. Moyed.

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