Studies on the Auxin Metabolism of Some Cereal Crops in Relation to Growth and Development II. Indoleacetic Acid-oxidase Inhibitors Contained in Wheat Seedlings

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Studies on the Auxin Metabolism of Some Cereal Crops in Relation to Growth and Development

II. Indoleacetic Acid-oxidase Inhibitors Contained in Wheat Seedlings*

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Summary

The inhibitors of IAA-oxidase contained in dark-grown wheat seedlings are studied in this paper.

Boiled water extract from dark-grown wheat seedlings showed an inhibiting effect on the IAA-oxidase activity when in higher concentration. It showed, however, a promoting effect when in lower concentration. Moreover, it showed a little interference with Salkowski's color reaction of IAA.

The inhibitors were extracted from the fresh materials with methanol, n-butanol, iso-propanol, acetone, acetonitrile or ethylacetate with ease. But with benzene, n-hexane or petroleum ether, extraction was difficult.

In order to identify the inhibitors, we have undertaken some experiments using paper chromatography and obtained the following results: these inhibitors showed positive color reactions with $FeCl_3$ and diazo (alkaline) reagents, but negative with Al^{+++} and diazo (acid) reagents. As a result, it is suggested that these inhibitors are phenolics excluding flavonoids and catechins.

From further studies using two-dimensional paper chromatography, it may be concluded that the IAA-oxidase inhibitors contained in dark-grown wheat seedlings are ferulic acid and other less active phenolcarboxylic acids.

Since 1953, many experiments with various plants have been conducted on the IAA-oxidase inhibitors contained in plant tissue. Gortner and Kent (1) first found these native inhibitors based on the phenomenon that they were not able to get the normal dilution curve with IAA-oxidizing enzyme preparation from pineapple. Although the following experiments have been reported, the physiological role of these substances is not yet fully understood: the increase of these inhibitors in GA-treated plants (2, 3), the relationship between the content of

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the inhibitors and the response to photoperiod (3, 4), the enhancement of inhibitor synthesis under red light and its reversal under far-red light (5), their relation to bolting and flowering in long-day plants (6), and the chlorogenic acid (a sort of IAA-oxidase inhibitor) synthesis in rice coleoptile under anaerobic conditions (7).

The authors reported in the previous paper (8) that the dark-grown wheat seedlings contained some IAA-oxidase inhibitors. The present paper is the continuation of the earlier study and deals with identifying these substances by using paper chromatography.

Material and Methods

Material

The wheat seedlings, AOBAKOMUGI variety, grown by the same method reported in the previous paper (8) were used.

Measurement of the Inhibiting Power of IAA-oxidation

Five ml of the reaction mixture containing 3 ml of McIlvaine's buffer solution (pH 5.0), 10^{-4}M of IAA, 0.5 ml of inhibitor solution, and 1 ml of the dialysed enzyme solution prepared from coleoptiles using the same method as in the previous paper (8) were incubated at 30°C in the dark, shaking 100 times per minute for 60 minutes. After incubation, the amount of IAA in the reaction mixture was determined by the same method used in the previous paper (8). The degree of inhibition was expressed as the percentage of inhibition to the control.

Paper Chromatography

a) One-dimensional paper chromatography. Samples were prepared as follows: the coleoptiles, plumules and roots were detached from the seedlings separately and 1 g of each material was extracted with 200 ml of absolute methanol at 5°C for 24 hours and then the residue was filtered off and re-extracted with a small quantity of hot methanol. Both extracts were combined and concentrated in vacuo. Then the residue was dissolved separately in 1 ml of hot water, 5 μl of this solution were used for the paper chromatographic analysis using Toyo No. 51 filter paper (2×40 cm) and a mixture of n-butanol, acetic acid and water (4:1:5 v/v upper layer) as the developing solvent.

b) Two-dimensional paper chromatography. Sample was prepared using the procedure shown in Fig. 1. Twenty μl of this sample were used for paper chromatographic analysis using Toyo No. 51 filter paper (40×40 cm) and a mixture of benzene, acetic acid and water (10:7:3 v/v water layer) as the first developing solvent plus a 2 percent formic acid as the second developing solvent. In the cases of both ferulic acid and p-cumaric acid, two spots for each substance were detected if formic was used as the developing solvent. It may be caused

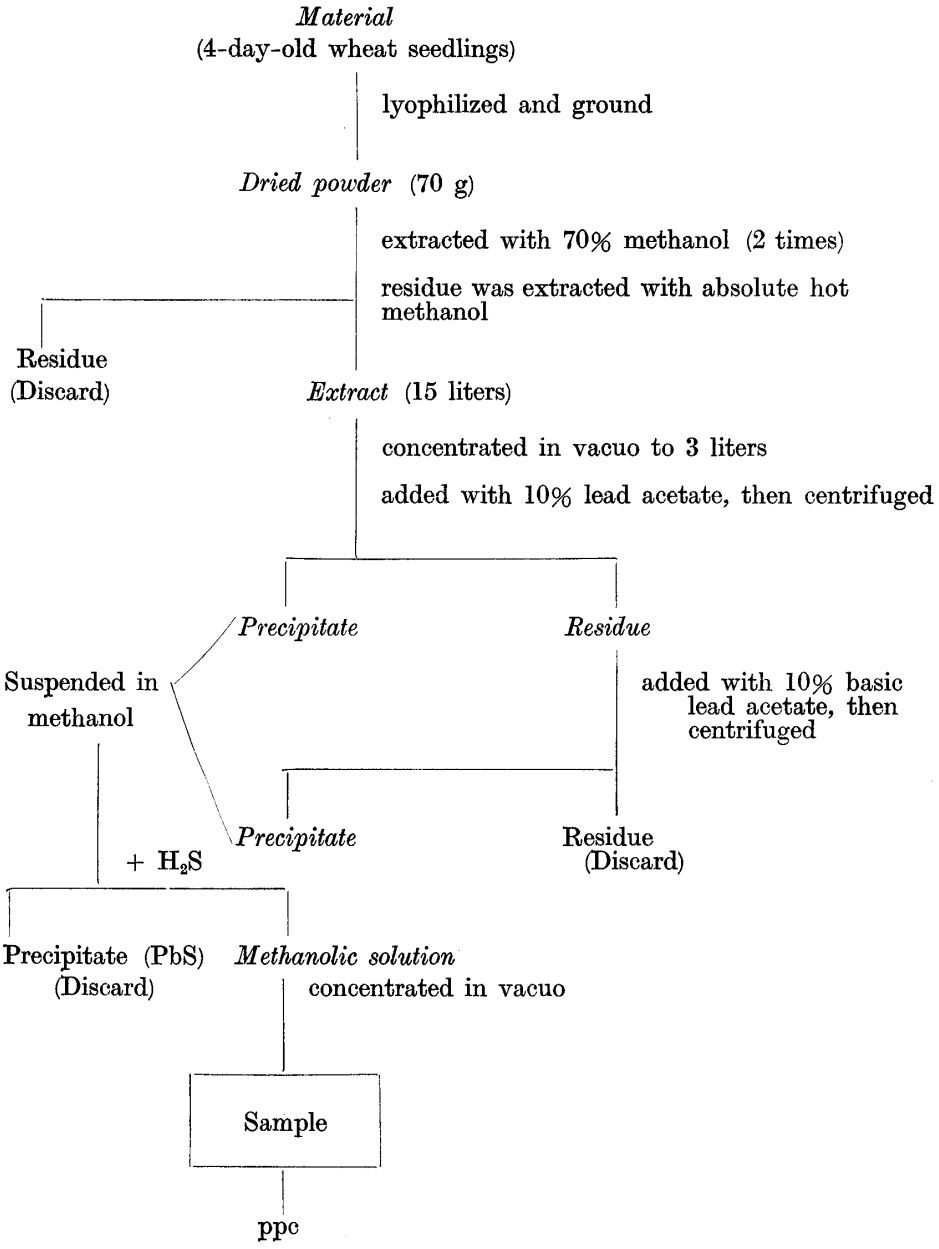

FIG 1. Extraction procedure of IAA-oxidase inhibitors from wheat seedlings.

by the separation of these substances into trans- and cis-form isomers as described by El-Basyonni and Towers (9).

Results and Discussion

Effect of the Boiled Water Extract of Wheat Seedlings on IAA-oxidation

The authors reported in the previous paper that the boiled water extract inhibited the IAA-oxidation. More detailed studies were carried out in this paper with various concentrations of the extract. As a result (Fig. 2), in the concentration where 1 ml of the extract was prepared from 50–200 mg (above

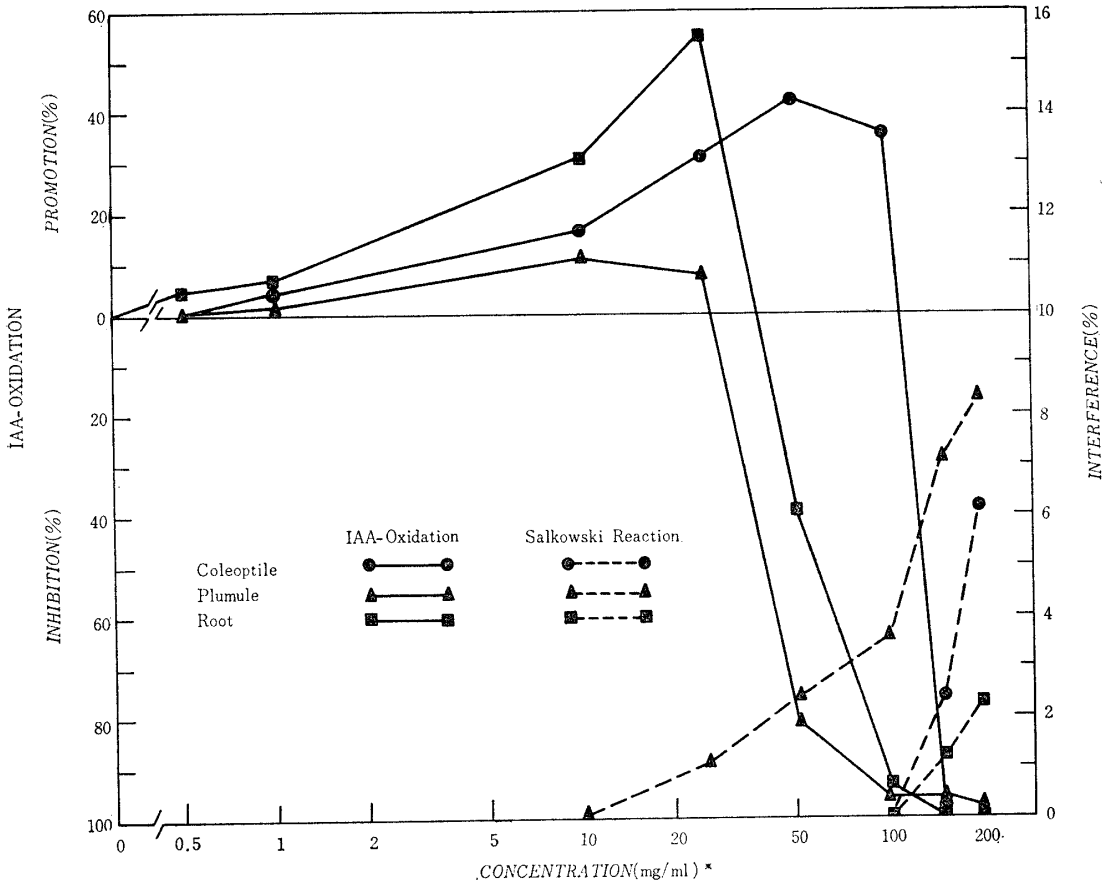

FIG. 2. Effect of boiled water extract in various concentrations from 5-day-old wheat seedlings on IAA-oxidation, and interference with Salkowski's color reaction by the extract.
* Concentration is represented as mg of fresh materials used for 1 ml of extract.

150 mg in coleoptiles) of fresh materials, it inhibited IAA-oxidation in all cases. It showed, however, a promoting effect in lower concentrations. From these results, it is thought that the IAA-oxidase inhibitors in wheat seedlings show the inhibiting effect only in higher concentrations and the promoting effect in lower concentrations. Moreover, the extract showed a slight interference with Salkowski's color reaction of IAA.

Extraction of IAA-oxidase Inhibitors with Various Organic Solvents

IAA-oxidase inhibitors in wheat seedlings were easily extracted with hot water. Besides, the extraction of the inhibitors from wheat seedlings were carried out with various organic solvents. That is, the coleoptiles, plumules and roots were detached separately from the seedlings and 1 g of each was extracted with 10 ml of various organic solvents at 5°C for 24 hours. The extract was filtered and centrifuged, then 5 ml of the supernatant was dried up in vacuo. The residue was dissolved in 2.5 ml of deionized water and 0.5 ml of this solution were used

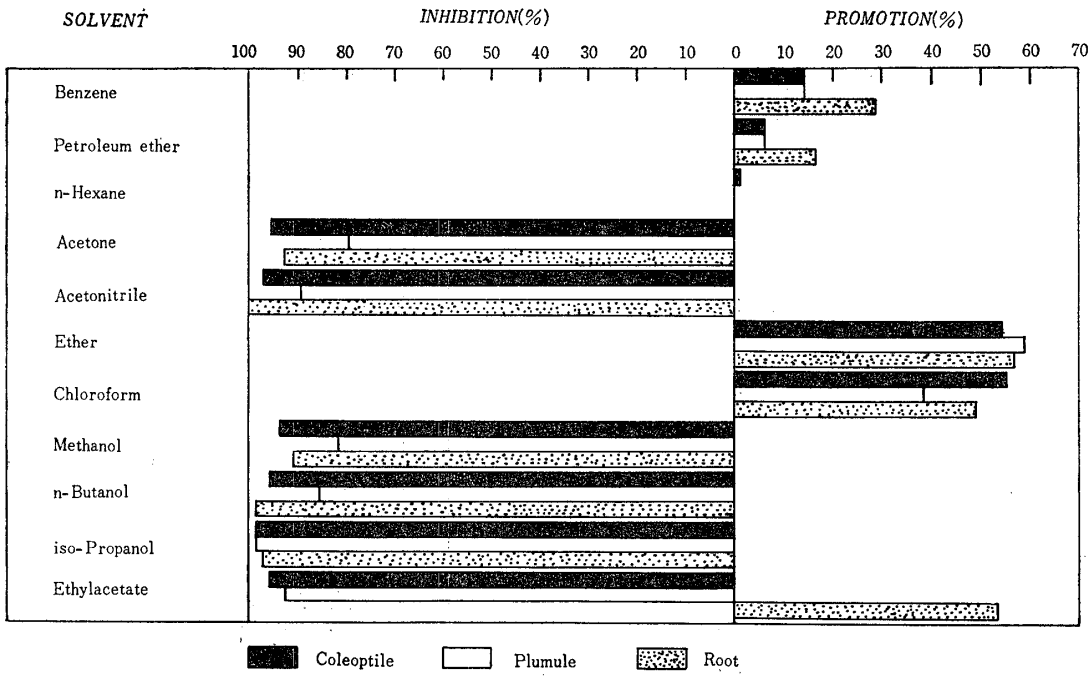

FIG. 3. Effect of extracts with various organic solvents on IAA-oxidase activity.

to test for IAA-oxidase inhibiting power. As a result (Fig. 3), it was observed that the inhibitors were easily extracted with methanol, n-butanol, iso-propanol, acetone, acetonitrile or ethyl acetate but that extraction was difficult with benzene, petroleum ether or n-hexane.

Paper Chromatographic Analysis

Methanolic extract of wheat seedlings was concentrated in vacuo and it was spotted and developed on the filter paper. After drying, the paper was cut into 10 pieces and they were added separately to the reaction mixture of IAA-oxidation for testing the inhibiting power. In addition to this, the papers were also tested for color reactions by using a 2 percent $FeCl_3$ methanolic solution, diazo (acid and alkaline) reagent and 1 percent of aluminium nitrate as the detecting reagent. As a result (Fig. 4), the inhibition has appeared on Rf 0.8–0.9 (markedly); on Rf 0–0.2 and Rf 0.3–0.5 (slightly). These regions showed positive color reactions with $FeCl_3$ and diazo (alkaline) reagent. However, they showed negative reactions with aluminium nitrate and diazo (acid) reagent. From these results, it was suggested that the IAA-oxidase inhibitors in wheat seedlings were phenolics except flavonoids and catechins.

With the large amount of lyophilized material, phenolics in wheat seedlings were identified by using two-dimensional paper chromatography. Detection of phenolics was carried out under UV-light aside from the use of some reagents mentioned above. As a result (Fig. 5), one substance having blue fluorescence and a few substances having violet fluorescence were detected under UV-light and they

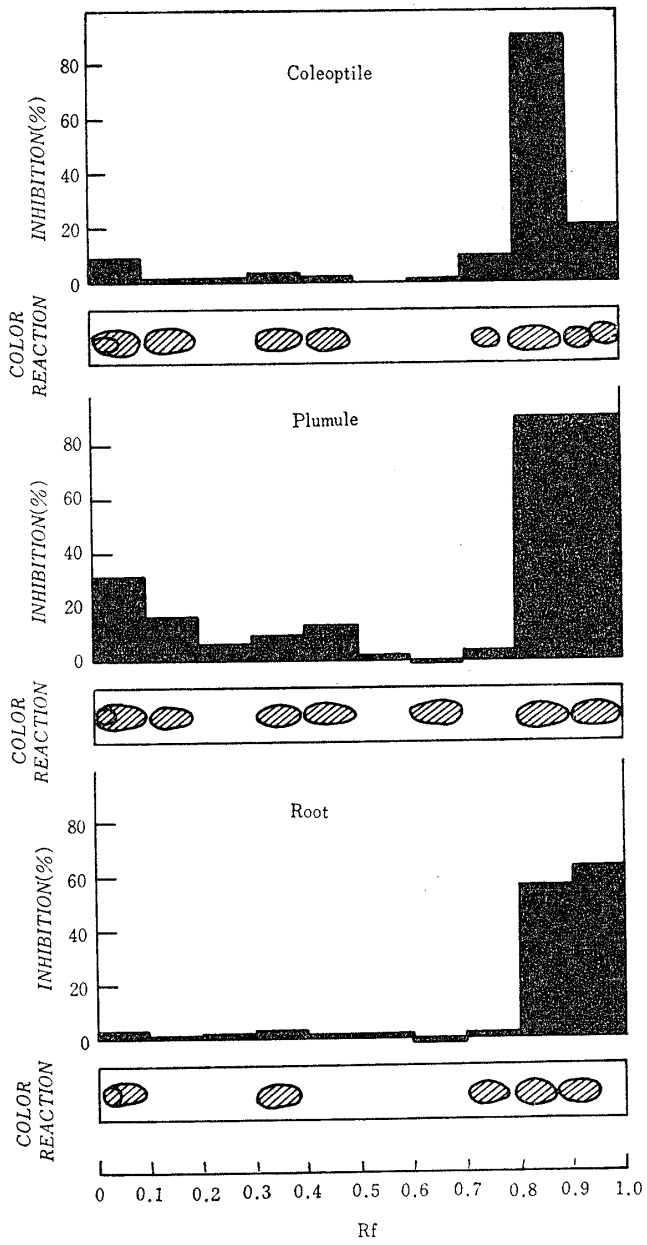

FIG. 4. Chromatograms of extracts from 4-day-old wheat seedlings, and histograms showing these effects on IAA-oxidation. $FeCl_3$ methanolic solution was used in color reaction.

were thought to be ferulic acid, vanillic acid, p-cumaric acid and syringic acid by comparing to test acids. Sinapic acid was not detected since the test substance was not available, although it was reported that wheat plant contained such (9).

Then, each spotted area was cut off separately; placed in a test tube with water so as to test its IAA-oxidase inhibiting power. As a result, the spots of ferulic acid showed strong inhibition of IAA-oxidation compared to other substances.

From the results mentioned above, it was considered that the IAA-oxidase

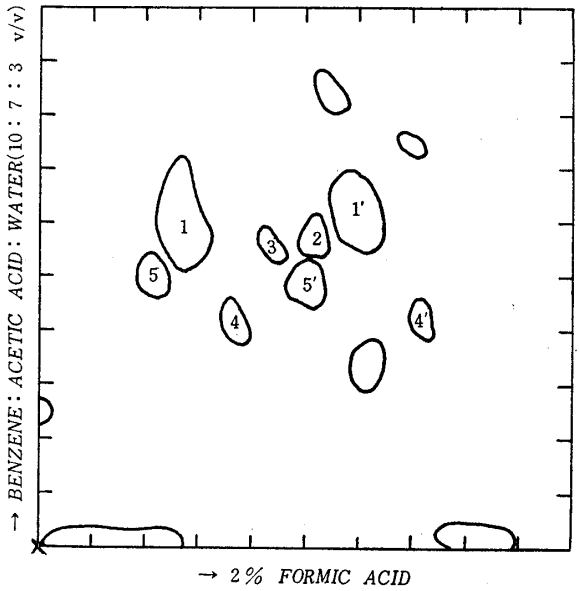

FIG. 5. Two-dimensional chromatogram of phenolics containd in 4-day-old wheat seedling.
Spots were detected under UV light.
1, 1′: Ferulic acid 2: Vanillic acid 3: Syringic acid 4, 4′: p-cumaric acid 5, 5′: Sinapic acid(?)

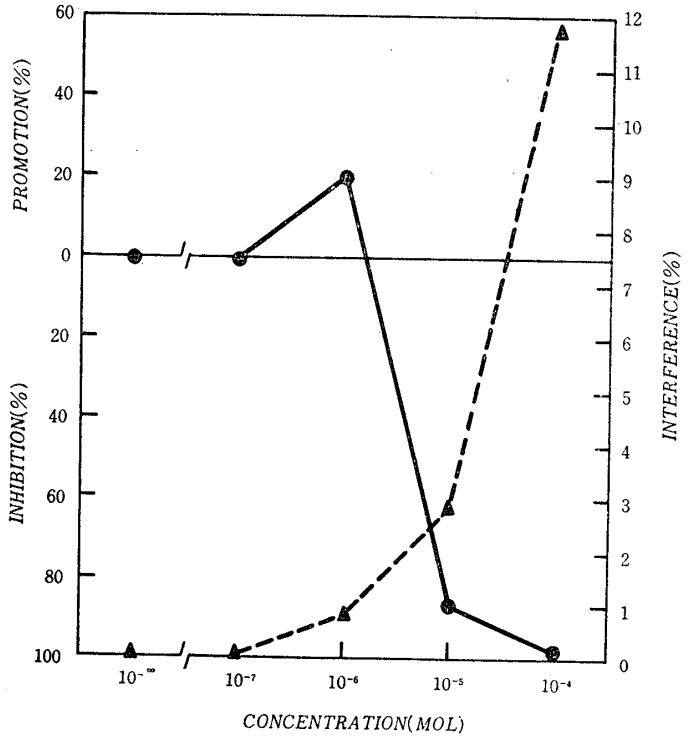

FIG. 6. Effects of ferulic acid in various concentrations on IAA- oxidation (•—•) and interference with Salkowski's color reaction. (▲---▲)

inhibitors contained in wheat seedlings were ferulic acid and other less active phenolcarboxylic acids.

Effect of Ferulic Acid on IAA-oxidase Activity

The effect of ferulic acid on IAA-oxidase and the Salkowski's color reaction was examined and the following results were obtained (Fig. 6). This substance showed an inhibiting effect when in higher concentrations and a promoting effect when in lower concentrations. These effects are almost the same as with the boiled water extract, although the extract showed a little higher promoting effect than ferulic acid and it seems to depend on p-cumaric acid which shows an extremely high promotion effect.

Acknowledgement

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