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Effects of Decursin and Decursinol on the Germination and Growth of Plants

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Decursin과 Decursinol이 植物의 生長과 發芽에 미치는 影響

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ABSTRACT

Biological activities of decursin and decursinol, natural coumarin derivatives, on the germination, growth and adventitious root formation of several plants were observed. In 10 ppm of decursin or decursinol, the growth of *Avena* coleoptile sections was inhibited, and the activity of IAA-oxidase was gradually enhanced by the increase of its concentrations. Inhibition effect on seed germination was observed from 100 ppm of each chemicals, and the activity of amylase in the germinating seeds was also gradually decreased. However, in the higher concentrations of decursin, the inhibited germination rate of wheat was slightly reduced. Decursin and decursinol also promoted the adventitious root formation in seeds of *Phaseolus vulgaris*.

INTRODUCTION

Decursin and decursinol, members of pyranocoumarin derivatives, are natural products found mainly in *Angelica*. Since they were isolated and identified (Chi, 1969; Konoshima *et al.*, 1968; Yen, 1970), some of pharmacological activities were studied (Chi and Kim, 1970). However, there is no report for the general biological activities of decursin and decursinol.

There are many reports on the biological activities of coumarin and its derivatives. The effects of coumarin on germination and growth of plants were widely studied. It has been demonstrated that coumarins have a pronounced inhibitory effect on root growth (Schreiner and Reed, 1907; Audus and Quastel, 1947; Goodwin and Taves, 1950; Goren and Tomer, 1971) and on seed germination (Hiromichi *et al.*, 1971). Coumarin was reported to inhibit the growth of sunflower (Knypl, 1964) and cucumber hypocotyl sections (Knypl and Rennert, 1967), while Neumann (1959) claimed that coumarin enhances the growth of excised sections of sunflower hypocotyl. On the other hand, Ihsan and Allon (1970) claimed that it inhibited the growth of *Hepaticae* at 100 mg/*l*, but enhanced at low concentrations.

The growth-retarding characteristic of coumarin moved several authors to investigate its effect on IAA-oxidase activity in the retarded tissues, which showed either an increase (Gortner *et al.*, 1958; Knypl and Rennert, 1967) or a decrease (Blumenthal-Goldschmidt, 1959) in IAA-oxidase activity. Scopoletin inhibited the activity of IAA-oxidase in tobacco (Sequeira, 1964) and both scopoletin and scopolin inhibited the initial IAA-oxidation by

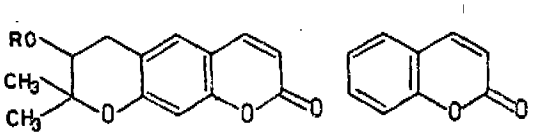
horseradish peroxidase (Schaeffer *et al.*, 1967). Seselin, a pyranocoumarin derivative, also inhibited radicle growth in seedlings of cucumber, lettuce, radish and wheat, and the activity of peroxidase and IAA-oxidase (Goren and Tomer, 1971). Besides the effect of coumarin on IAA-oxidase, Khan(1969) reported that coumarin almost completely inhibited α-amylase synthesis in barley seeds. Others examined the effect of coumarin on cellulose synthesis in azuki bean epicotyl sections (Hogetsu *et al.*, 1974).

The effects of coumarin on germination and growth are still not clear despite a long series of investigations (Schreiner and Reed, 1907, Audus and Quastel, 1947; Goodwin and Taves, 1950; Goren and Tomer, 1971; Svensson, 1971). Detailed investigations on the functioning mechanism of coumarin and its derivatives are being widely carried out for this reason (Svesson, 1971: 1972).

Basu (1972) examined that indole, α-naphthol, pyrogallol, coumarin and salicylic acid interacted with the auxins, IAA, NAA and 2,4—D supplied to the basal ends of cuttings of *Phaseolus vulgaris*, giving synergistic or antagonistic effects in root formation.

Paupardin and Tizio (1970) reported that certain phenolic compounds are active in the tuberization of potato sprout sections *in vitro*. By other worker, coumarin stimulated the tuber initiation of excised shoots of *Solanum tuberosum* (Stallknecht, 1972).

As mentioned above, coumarin and some of its derivatives have been subjected to active investigations. However, the biological activities of decursin and decursinol are still not known, except for some general pharmacological activities of them. For this reason, attempts were made in the present study to investigate the effect of decursin and decursinol on germination, growth and adeventitious root formation. The correlations of growth and IAA-oxidase and of germination and amylase activity were also studied.



R=H: Decursinol
R=OC·CH·C(CH3)2 : Decursin

Coumarin

MATERIALS AND METHODS

Materials

Seeds of *Avena sativa* L. cv. Victory were kindly provided by the Crop Experiment Station Office of Rural Development. Other seeds, wheat, lettuce (Grand Rapids), tomato (Bogwon No. 2), cucumber (Shinrok Dadagi), Chinese cabbage (Seoul) and French bean (*Phaseolus vulgaris* L.) were commercially purchased. All seeds used in this experiment were sterilized by dipping in 0.1% $HgCl_2$ for 10 min and washed with distilled water prior to germination. Decursin and decursinol were isolated and purified from *Angelica gigas* by etheral extraction and silicagel chromatography (Konoshima *et al.*, 1968).

Straight growth test and IAA-oxidase assay

The germinated *Avena* seeds were grown in the dark at room temperature for 72 hr. The coleoptiles of seedlings were cut 5 mm from the tips and then 6 mm sections were made for test. Each of 20 coleoptile sections were incubated in petri dishes containing test solutions for 20 hr at 27°C in darkness. All solutions contained 14.0 ml of phosphate buffer (1/60 M, *p*H 6.2, containing 2 % sucrose), 0.5 ml of 10^{-4}M IAA and 0.5 ml of requisite concentration of decursin, decursinol or coumarin. These 3 compounds were eliminated from the control solution.

After incubation, each of coleoptile sections was measured its length. Ten sections of them were measured their fresh weight, washed with distilled water and ground in a homogenizer with 10 ml of chilled 0.1 M phosphate buffer, *p*H 6.0 (Goren and Tomer, 1971) in an ice bath. The homogenate was centrifuged at 1,000g for 30 min and the supernatant was used for the test of IAA-oxidase activity (Whitmore, 1971). Reaction mixture contained 0.025 ml of 1 mM 2,4-dichlorophenol, 1ml of 1 mM IAA in 0.5 mM $MnCl_2$, 3.0 ml of 0.02 M KH_2PO_4 and 1.0 ml of the enzyme solution (Whitmore, 1971). The mixture was incubated in water bath at 30°C for 2 hr. One ml of the reaction mixture was mixed with 2 ml of Salkowski reagent (Gordon and We-

ber, 1951) and the mixed solution was allowed to complete the reaction for 1 hr. The extinction of solutions was measured with a Beckman-DU spectrophotometer at 530 nm.

Germination test and Amylase assay

Seeds of lettuce, tomato, cucumber, Chinese cabbage and wheat were placed in petri dishes containing test solutions and allowed to germinate for 2 days in darkness. But tomato seeds were germinated after 10 days. All solutions contained 8.7 ml of distilled water and 0.3 ml of requisite concentration of decursin, decursinol or coumarin. Control solution contained 9.0 ml distilled water only. After the incubation, the number of 'germinated seeds' was counted. A 'germinated seed' was defined as one whose radicle protruded at least 3 mm (Marchaim *et al.*, 1972).

For amylase activity test, roots and shoots from 5 wheat seedlings were removed, and ground in a homogenizer with 50ml of 0.016 M sodium acetate buffer, *p*H 4.8, for 10 min (Gepstain and Ilan, 1974). The homogenate was centrifuged at 2000×*g* for 10 min and the supernatant was used as an enzyme solution. Amylase activity was measured as follows: Half milliliter of enzyme solution was added to 0.5 ml of soluble starch reagent (Bilderback, 1971) and incubated for 10 min at 30°C in water bath. The reaction was stopped by the addition of 2 ml iodine reagent (Bilderback, 1971). The optical density of starch-iodine complex solution was measured at 620 nm with a spectrophotometer. Results were expressed as amounts of hydrolyzed starch per fresh weight of seeds (μg/mg) in 10 minutes.

Root initiation test

Seeds of French bean were soaked in water for 24 hr and allowed to germinate in wet thin cotton layer in the light for 9–10 days at 27°C. With the seedlings of 10–12 cm tall, 8–10 cm long cuttings with 5 cm hypocotyl portions were made and cotyledons were also excised from the cuttings.

A series of beakers, each containing 9 ml of requisite concentration of decursin, decursinol, or coumarin in distilled water, was employed. Ten cuttings were placed in each beaker and allowed to stand for 24 hr to take up the chemicals. The treated cuttings were transferred thereafter to the beakers containing water only. The water was changed everyday for a week. Numbers of adventitious roots produced per cuttings were counted (Basu, 1972).

RESULTS

In the *Avena* straight growth test, both of decursin and decursinol inhibited the IAA-induced growth of all coleoptile sections tested in a concentration range of 10–100 ppm. Decursin, decursinol and coumarin at 100 ppm inhibited the growth by 18.54, 28.84 and 49.44 per cent, respectively. Thus, decursinol showed more inhibitory effect than decursin (Table 1).

The effects of decursin, decursinol and coumarin on the activity of IAA-oxidase were shown in Fig. 1. It is evident that the increased concentrations of compounds enhanced the activity of IAA-oxidase. Increased IAA-oxidase activity was corresponding to accompanying growth retardation (Table 1). Decursinol had more synergistic effect to IAA-oxidase than decursin, while coumarin was much more effective.

Decursin (100–500 ppm), decursinol (100–500

Table 1. Effects of decursin, decursinol and coumarin on the growth of *Avena* coleoptile sections

Treatment	Conc ppm	Increase in length of the sections	Inhibition rate, %
Control	none	5.34±0.84	—
Decursin	10	5.03±1.28	5.81
	50	4.58±0.56	14.23
	100	4.35±0.84	18.54
Decursinol	10	5.00±0.54	6.37
	50	4.37±0.62	18.16
	100	3.80±0.86	28.84
Coumarin	10	4.28±0.86	19.85
	50	3.38±1.08	36.70
	100	2.70±0.83	49.44

Twenty coleoptile sections were incubated for 20 hr at 27°C in 15 ml of the solution in darkness.

ppm) and coumarin (10-100 ppm) inhibited the germination of all the seeds tested, except for cucumber and Chinese cabbage in case of decursin (Table 2). Certainly, decursinol was more effective than decursin, though was weaker than coumarin, as in *Avena* straight growth test (Table 1). However, the inhibiting effect of decursin on the germination of wheat seeds rather decreased at the concentration range of 300-400 ppm (Fig. 4).

Figure 2 shows the effects of decursin, decursinol and coumarin on the activity of amylase. Certainly, when the concentration of these chemicals was increasd, they inhibited the activity of amylase gradually. Inhibition of amylase activity was found to accompany with the retardation of germination (Table 2). One mg tissue of wheat seedlings treated with 100 ppm of decursin, decursinol or coumarin could hydrolyze 12.46, 9.85 and 6.24 μg each of soluble starch in 10 min, respectively. The antagonistic effect to amylase activity increased in the order of decursin, decursinol and coumarin. Figure 3 shows mean number of adventitious roots initiated in per cuttings at the presence of decursin, decursinol or coumarin. These compounds promoted root formation at a concentration range of 50-100 ppm. The effects of coumarin derivatives on adventitious root formation showed the same tendency as in growth and germination test (Tables 1 and 2).

DISCUSSION

From the results of this experiment, decursin and decursinol can be considered as inhibitors on growth and germination in plants (Tables 1 and 2), like coumarins (Goren and Tomer, 1971; Hiromichi *et al.*, 1971; Svensson, 1971; Miller *et al.*, 1975). It is noteworthy that decursin and decursinol, like coumarin, antagonized with the IAA-induced elongation of *Avena* coleoptile sections (Table 1).

In a condition of fixed IAA concentration *in vitro*, the growth of plant was mainly controlled by the activity of IAA-oxidase. However, in cells, IAA-oxidase was determined by two ways; One is the production rate of *de novo* synthesis of enzyme proteins. The other is to measure the changes of the enzyme activity. Coumarin and other growth retardants increased the activity of IAA-oxidase and this results were correlated with the inhibition of the growth (Gortner *et al.*, 1958; Halevy, 1963; Miller *et al.*, 1959; Fig. 1). There is an evidence that these chemicals including coumarin and seselin did not induce the *de novo* synthesis of IAA-oxidase, but acted as the enzyme cofactor (Goren and Tomer, 1971). Goren and Tomer (1971) had thought xanthyletin also to be a cofactor, because it was a linear isomer of seselin. So it can be said that decursin and decursinol are also IAA-oxidase cofactors.

In seeds, prior to the initiation of germination processes, it is necessary that the activation of certain enzymes should occur and followed by the synthesis of hydrolytic enzymes. Particulary, in wheat seeds, amylase (hydrolytic enzymes of carbohydrates) activity could control the rate of germination (Khan, 1969). Besides this experiment, coumarin decreased the enzyme activity and inhibited the synthesis of α-amylase (Khan, 1969). Seselin also inhibited GA_3-induced sugar release in barley endosperm (Goren and Tomer, 1971). It

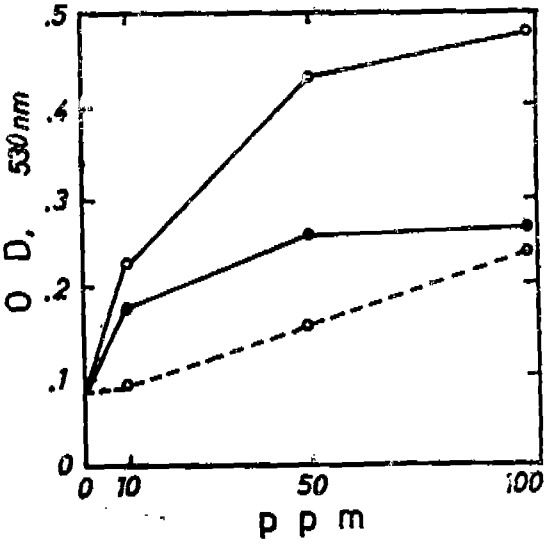


Fig. 1. Comparison of decursin, decursinol and coumarin on IAA oxidase activity in *Avena* seedlings. Decursin; ○···○, decursinol; ●—●, coumarin; ○—○.

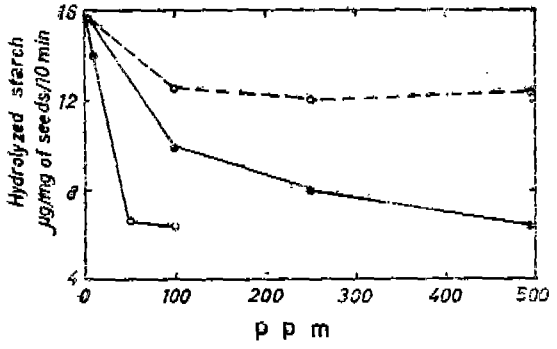


Fig. 2. Effect of decursin, decursinol and coumarin on amylase activity in wheat seedlings. Decursin; ○···○, decursinol; ●—●, coumarin; ○—○.

seemed that decursin and decursinol could be inhibitors to the synthesis of enzymes, including amylase.

However, the increased concentration of decursin does not correlate with the activity of amylase in wheat seeds (Fig. 2) and the germination rates of wheat seeds were not proportinately affected in the presence of decursin. (Fig. 4). Further work should be done for these phenomena. Several compounds including sesquiterpene lactones. (Yamaki *et al.*, 1966; Shibaoka *et al.*, 1967) and coumarin (Basu, 1972) were themselves significant stimulators of adventitious root formation in plants when applied it alone. And other compounds had chemically related structures with coumarin also showed many kinds of biological activities in different speicies. There is an evidence that coumarin stimulated ethylene synthesis, with a suggestion that ethylene mediated the inhibitory actions (Morgan and Powell, 1970). There are indirect evidences that ethylene could decrease auxin supply (Morgan and Gausman, 1966; Scott, 1972). If ethylene can really change endogenous auxin concentration in tissues, this would be a factor to differentiate the initiation of adventitious root. It can be suggested that decursisn and decursinol, like coumarin, might change the concentration of ethylene followed by the change in auxin concentration, which initiates the formation of adventitious root.

In a view point of their chemical structures, all of the 3 compounds have a lactone group with an endo-unsaturated double bond. Such compounds have ordinarily specific biological sctivities (Jones

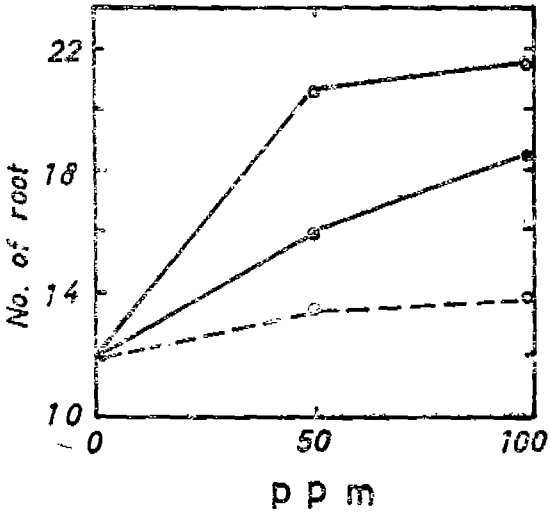


Fig. 3. Mean numbers of adventitious roots of French bean cuttings in the presence of decursin, decursinol and coumarin. Decursin; ○···○, decursinol; ●—●, coumarin; ○—○.

Table 2. Effects of decursin, decursinol and coumarin on the germination rates of the seeds

	Inhibition rate, %								
	Decursin			Decursinol			Coumarin, ppm		
Seeds	100	250	500	100	250	500	10	50	100
Lettuce	13.3	20.0	23.3	21.2	30.0	44.4	80.0	93.3	100
Wheat	30.6	41.2	27.1	25.9	51.8	77.6	11.8	64.7	76.5
Cucumber	10.0	10.0	5.3	—	17.5	20.0	17.5	37.5	42.5
Tomato	—	9.3	20.0	14.7	57.3	80.0	36.0	84.0	84.0
Chinese cabbage	2.1	3.1	5.2	6.3	21.9	33.3	6.3	17.7	33.3

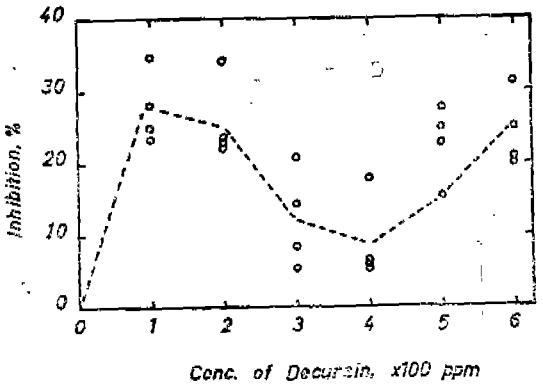


Fig. 4. Effect of Decursin on the germination of wheat seeds.

and Young, 1968; Kwon and Woo, 1975; Shibaoka *et al.*, 1967). However, when such a compound is modified on its double bond by saturation, it usually becomes a biologically inert compound. In cases of decursin and decursinol, each of them have a lactone group which has the unsaturated double bond. Though the mechanism of adventitious root formation by the chemicals could not be explained the reason of promoted root formation by decursin and decursinol could be entirely depended on their chemical structures.

By the results of this experiment, biological activities of decursin were always lower than decursinol. It could be explained with the fact that decursin has an isopentenyl group because most of biologically active compounds containing lactones were reduced their activities mainly when their structures were modified with isopentenyl group (Komissarenko *et al.*, 1971), even if their lactone groups were not affected.

Regarding all the results above, a conclusion could be made as follows: Decursin and decursinol inhibit the growth of *Avena* coleoptile sections by acting as IAA-oxidase cofactors. The decrease in the germination rate of wheat seed by decursin and decursinol is supposed to be the result of their inhibition on the activity and/or synthesis of amylase. The mode of action of decursin and decursinol on adventitious root formation, too, is assumed to be the same as that of the other chemicals similar in chemical structure. As in a case of coumarin, the change in intracellular auxin concentration is thought to be the reason of promoting adventitious root formation, although no confirmation was made in the present study. One unexpected result, that the germination rate of wheat seed was not inhibited in proportion to decursin concentration, was observed, giving rise to the necessity of further investigation.

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