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Synthesis and Bleaching Activity of 1–Ethyl– and 1–Propyl–5–Substituted Imidazoles

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A series of 1–propyl–5–substituted imidazoles were synthesized and evaluated for bleaching activity in 4–day–old lettuce seedlings. Among the tested compounds, 5–(2–naphthyl) **9**, 2–phenethyl **15** and cinnamyl **18** analogs brought about bleaching injury at only 5 ppm. Contents of pigments were determinated in the radish seedings treated with some 1–propyl–5–substituted imidazoles. Compound **18** at 50 ppm decreased chlorophyll and total carotenoids contents in the radish seedings to less than 15% and 25%, respectively, of that in the control.

INTRODUCTION

In the recent year, several herbicides that cause bleaching in plant tissue have been well reviewed. The term of bleaching refers to a decrease in the amount of pigments after a certain period of growth in the presence of a herbicide as compared to an untreated control. These herbicides inhibit the biosynthesis of essential components of the photosynthetic apparatus such as pigments of carotenoids and chlorophyll in the photosynthetic membrane. Most of the bleaching herbicides, such as fluridone (Schoder *et al.*, 1992) and norflurazon (Snadmann *et al.*, 1992), interfere with phytoene desatulation step in β–carotene biosynthesis, and bring about inhibition of colored carotenoids synthesis with accumulation of phytoene, and blight the plant finally (Sandmann *et al.*, 1991). A variety of chemical unrelated compounds have been found to show bleaching activity against many higher plants and green algae.

We have reported that 5–phenyl–1–propylimidazole or 5–(4–benzyloxyphenyl)–1–propylimidazole had bleaching activity for the lettuce seedlings, and reduced chlorophyll and total carotenoids (Yamada *et al.*, 1992). The mode of action of these 1,5–disubstituted imidazoles with bleaching activity is unclear, but it is unique that two types of 1,5–disubstituted imidazoles which have different substituents (5–phenyl analog and 5–(4–benzyloxyphenyl) analog) cause distinct bleaching. There is a possibility that two types of imidazoles affect on the different target sites to cause bleaching, and 1,5–disubstituted imidazoles with other substituents at 5–position of imidazole rings have strongly bleaching activity. In the present paper, we describe that detailed structure–bleaching activity relationships of 1–ethyl– and 1–propyl–5–substituted imidazoles and the mode of action of these imidazoles, mainly their effect on carotenoids biosynthesis.

MATERIALS AND METHODS

1. Chemicals

All melting points (mp.) are uncorrected. ^{1}HNMR spectra were determined with a JEOL FX-100 spectrometer, using tetramethylsilane as an internal standard, and samples were prepared in deutero-chloroform.

Compounds **1-3**, **13** and **25** were synthesized according to the precedure reported previously (Yamada *et al.*, 1992).

1-Propyl-5-(4-stilbenyl)imidazole **(4)** To a solution of 6.15 g (0.0166 mol) of *N*-(4-stilbenyl-α-tosylstyryl)formamide **(26)** in 50 ml of dry dimethoxyethane (DME) under nitrogen was added drop by drop 6.1 g of phosphorus oxychloride in 10 ml of DME at -15 °C. After stirring for 1 hr at room temperature, the mixture was poured into ice-cooled water (200 ml) and extracted with dichloromethane. The dichloromethane solution was washed with brine, dried over sodium sulfate, and concentrated. The residue was dissolved in 50 ml of methanol, and to the mixture was added 1.83 g of *n*-propylamine and 16.8 g of triethylamine. After stirring for 1 day at room temperature, ice-water was added to the mixture, and the product was extracted with diethylether. The diethylether solution was washed with brine, dried over sodium sulfate, and concentrated. The recrystallization of the reside from methanol and *iso*-propylether afforded 1.43 g (0.0050 mol) of **4**. Yield 29.8%. mp.136 °C. ^{1}H-NMR ($CDCl_3$) δ : 0.84 (3H, t, J=7 Hz), 1.45-1.86 (2H, m), 3.93 (2H, t, J=7 Hz), 7.02-7.61 (13H, m).

The starting material **26** was prepared as follow.

N-(4-Stilbenyl-α-tosylstyryl)formamide **(26)** A solution of 3.7 g of tosylmethylisocyanide (Tos MIC) in 50 ml DME was added dropwise to a stirred suspension of 3.02 g (0.0269 mol) of potadium *tert*-butoxide under nitrogen at -35 °C. To the mixture was added drop by drop a solution of 4-stilbenylcarboaldehyde (4.0 g, 0.0192 mol) in 50 ml DME at -50 °C. After stirring for 30 min at -40 °C, the mixture was pouered into 300 ml ice-water and acidefied with acetic acid. The precipitate was collected by filtration, washed with water, and recrystallized from ethyl acetate and toluene. 6.59 g (0.0163 mol) of **26** was obtained. Yield 85.0%. ^{1}H-NMR ($CDCl_3$) δ : 0.84 (3H, t, J=7 Hz), 1.45-1.86 (2H, m), 3.93 (2H, t, J=7 Hz), 7.02-7.61 (13H, m).

1-Propy-5-(4-pyridyl)imidazole **(5)** A solution of 4-pyridinecarboxyaldehyde (3.21 g, 0.03 mol), *n*-propylamine (2.66 g, 0.045 mol,) and anhydrous magnesium sulfate (10.8 g, 0.09 mol) in 50 ml of dichloromethane was stirred at reflux for 3 hr. After filtrating of magnesium sulfate, the filtrate was concentrated. To the residue in 50 ml methanol was added Tos MIC (7.03 g, 0.036 mol) and anhydrous potassium carbonate (8.29 g, 0.06 mol). After stirring at reflux for 3 hr, the solution was concentrated, and 50 ml of water was added to the concentrate. The solution was extracted with ethyl acetate two times. The ethyl acetate was washed by water and brine, dried over sodium sulfate. Concentration of column chromatography on silica gel afforded 0.013 g of pure **5**. Yield 0.23%. ^{1}H-NMR ($CDCl_3$) δ : 0.96 (3H, t, J=7 Hz), 1.52-1.94 (2H, m), 3.60 (2H, d, J=7 Hz), 7.14-7.64 (3H, m), 8.23 (1H, s), 8.50-8.70 (2H, m).

Compounds **6-24** were prepared in the same manner as **5** with use of a corresponding aldehyde, instead of 4-pyridinecarboxyaldehyde.

1-Propy-5-(3-pyridyl)imidazole **(6)** Yield 4.1%. ^{1}H-NMR ($CDCl_3$) δ : 0.85 (3H, t,

J=7 Hz), 1.45–1.87 (2H, m), 3.93 (2H, t, J=7 Hz), 7.01–7.76 (4H, m), 8.48–8.66 (2H, m).

1-Propy-5-(2-pyridyl)imidazole **(7)** Yield 2.7%. ^{1}H-NMR ($CDCl_3$) δ : 0.83 (3H, t, J=7 Hz), 1.38–1.86 (2H, m), 3.91 (2H, t, J=7 Hz), 7.02 (1H, s), 7.04–7.39 (4H, m), 7.52 (1H, s).

5-(1-Naphthyl)-1-propylimidazole **(8)** Yield 7.2%. ^{1}H-NMR ($CDCl_3$) δ : 0.69 (3H, t, J=7 Hz), 1.15–1.81 (2H, m), 3.66 (2H, t, J=7 Hz), 7.10 (1H, s), 7.27–7.95 (8H, m).

5-(2-Naphthyl)-1-propylimidazole **(9)** Yield 5.7%. ^{1}H-NMR ($CDCl_3$) δ : 0.79 (3H, t, J=7 Hz), 1.38–1.83 (2H, m), 3.95 (2H, t, J=7 Hz), 7.12 (1H, s), 7.29–7.92 (8H, m).

5-(3,4-Methylenedioxypheyl)-1-propylimidazole **(10)** Yield 24%. ^{1}H-NMR ($CDCl_3$) δ : 0.84 (3H, t, J=7 Hz), 1.44–1.86 (2H, m), 3.84 (2H, t, J=7 Hz), 4.27 (1H, s), 6.64–6.84 (3H, m), 6.84 (1H, s), 7.52 (1H, s).

5-(3,4-Ehylenedioxyphenyl)-1-propylimidazole **(11)** Yield 7.6%. ^{1}H-NMR ($CDCl_3$) δ : 0.84 (3H, t, J=7 Hz), 1.44–1.86 (2H, m), 3.84 (2H, t, J=7 Hz), 4.27 (4H, s), 6.68–7.01 (4H, m), 7.47 (1H, s).

5-(3-Indolyl)-1-propylimidazole **(12)** Yield 53%. mp.112 °C. ^{1}H-NMR ($CDCl_3$) δ : 0.83 (3H, t, J=7 Hz), 1.38–1.86 (2H, m), 3.91 (2H, t, J=7 Hz), 7.02 (1H, s), 7.04–7.39 (4H, m), 7.52 (1H, s).

5-(α-Methylbenzyl)-1-propylimidazole **(14)** Yield 2.2%. ^{1}H-NMR ($CDCl_3$) δ : 0.74 (3H, t, J=7 Hz), 1.18–1.67 (5H, m), 3.48 (2H, t, J=7 Hz), 3.96 (1H, q, J=7 Hz), 6.88–7.42 (7H, m).

5-(2-Phenethyl)-1-propylimidazole **(15)**. Yield 11%. ^{1}H-NMR ($CDCl_3$) δ : 0.83 (3H, t, J=7 Hz), 1.38–1.86 (2H, m), 3.91 (2H, t, J=7 Hz), 7.02 (1H, s), 7.04–7.39 (4H, m), 7.52 (1H, s).

5-(3-Phenylpropyl)-1-propylimidazole **(16)** Yield 12%. ^{1}H-NMR ($CDCl_3$) δ : 0.97 (3H, t, J=7 Hz), 1.39–1.92 (2H, m), 2.35 (2H, t, J=7 Hz), 2.95 (2H, t, J=7 Hz), 4.01 (2H, t, J=7 Hz), 6.71 (1H, s), 6.83– 7.32 (5H, m).

5-(Phenoxymethyl)-1-propylimidazole **(17)** Yield 8.5%. ^{1}H-NMR ($CDCl_3$) δ : 0.82 (3H, t, J=7 Hz), 1.40–1.84 (2H, m), 3.89 (2H, t, J=7 Hz), 4.01 (2H, s), 6.84–7.60 (6H, m).

Phenoxyacetoaldehyde used as starting material was prepared as follow.

Phenoxyacetoaldehyde **(27)** The reaction mixture of phenol (3.76 g, 0.04 mol) and bromoacetoaldehyde dimethyl acetal (7.65 g, 0.045 mol) and potassium carbonate (14.65 g, 0.008 mol) in dimethylformeamide was heated at 120 °C for 8 hr with stirring. After adding the water to the mixture, the solution was extracted with dichloromethane. The dichrorometane was treated with 5% NaOH solution and washed with water and brine, dried over sodium sulfate, and concentrated. After purification by column chromatography on silica gel, 6.94 g (0.038 mol) of phenoxyacetoaldehyde dimethyl acetal was obtained. [Yield 95%. ^{1}H-NMR ($CDCl_3$) δ : 3.38 (6H, s), 3.92 (2H, d, J=7 Hz), 4.64 (1H, t, J=7 Hz), 6.80–7.34 (5H, m)]. The solution of phenoxyacetoaldehyde dimethyl acetal (3.00 g, 0.016 mol) in 60 ml of acetic acid and ethanol (1:1) was warmed at 80 °C with stirring for 8 hr. After concentrating, water was added to the residue, and the mixture was extracted with diethylether. The organic solution was treated with 5% NaOH solution, and washed with water and brine, dried over sodium sulfate, and concentrated. Concentration followed by column chromatography on silica gel give pure **27** (1.14 g, 0.0084 mol). Yield 52%. ^{1}H-NMR ($CDCl_3$) δ : 4.62 (2H, s), 6.72–7.48 (5H, m). 9.88 (1H, s)

1-Propyl-5-strylimidazole **(18)** Yield 51%. mp.82°C. ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, t, J=7 Hz), 1.38–1.86 (2H, m), 3.91 (2H, t, J=7 Hz), 6.85 (2H, d, J=7 Hz), 7.16–7.50 (5H, m).

5-(4-Chlorostyryl)-1-propylimidazole **(19)** Yield 1.8%. mp.68°C. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, t, J=7 Hz), 1.53–1.96 (2H, m), 3.80 (2H, t, J=7 Hz), 6.11–6.58 (2H, m), 6.84 (2H, s), 7.10–7.32 (4H,m).

4-Chlorocinnamaldehyde used as starting material was prepared as follow.

4-Chlorocinnamaldehyde **(28)** To a solution of ethyl 4-chlorocinnamate (4.98 g, 0.024 mol) in dry dichloromethane at −70°C under nitrogen gas was added the toluene solution of diisobutylaluminium hydride (0.93 M solution; 38 ml, 0.0035 mol) drop by drop. After stirring for 1 hr at −70°C, to the was added saturated ammonium chloride solution. The solution was filtrated and concentrated. Purification by column chromatography on silica gel gave 2.77 g (0.016 mol) of 4-chlorocinnam alchol. [Yield 69%. ^{1}H-NMR ($CDCl_3$) δ: 1.89–2.18 (1H, brode), 4.26 (2H, d, J=7 Hz), 6.10–6.60 (2H, m), 7.14 (4H, s)]. To a solution of oxalyl chloride (4.17 g, 0.033 mol) in dry dichloromethane at −70°C under nitrogen gas was added the mixture of dimethyl sulforxide (3.85 g, 0.049 mol) slowly. After stirring for 10 min, the solution of 4-chlorocinnam alcohol (2.77 g, 0.016 mol) in dry dichloromethane was added to the mixture dropwise. After stirring for 30 min at −70°C and for 1 hr at −40°C, triethylamine (16.6 g, 0.164 mol) was added, and the mixture was stirred for 1 hr at 0°C. After quenching with water, the mixture was extracted with dichloromethene, the dichloromethen was treated with 5% sodium hydrocabonate and washed water and brine, and dried over sodium sulfate. After concentrating of dichloromethene, the residue was purified by column chromatography on silica gel. 0.84 g (0.0055 mol) of pure **28** of was obtained. Yield 34%. ^{1}H-NMR ($CDCl_3$) δ: 6.52–5.78 (2H, m), 7.02–7.68 (4H, m), 9.72 (1H, d, J=7 Hz).

5-(3-Chlorostyryl)-1-propylimidazole **(20)** Yield 0.77%. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=7 Hz), 1.58–1.88 (2H, m), 3.80 (2H, t, J=7 Hz), 6.11–6.58 (2H, m), 6.90 (2H, s), 7.10– 7.41 (5H, m).

3-Chlorocinnamaldehyde used as starting material was prepared in the same way as **28** with use of ethyl 3-chlorocinnamate instead of ethyl 4-chlorocinnamate.

5-(2-Phenyl-1-methyl-ethenenyl)-1-propylimidazole **(21)** Yield 6.2%. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7 Hz), 1.56–1.98 (2H, m), 2.20 (3H. s), 3.94 (2H, t, J=7 Hz), 6.55 (1H, s), 6.92–7.50 (7H, m).

5-(2-Furan-trans-ethenenyl)-1-propylimidazole **(22)** Yield 13%. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, t, J=7 Hz), 1.54–1.98 (2H, m), 1.89 (3H, t, J=7 Hz), 6.23–6.42 (2H, m), 6.70 (2H, s), 7.24 (1H, s) 7.30–7.44 (4H, m).

5-[2-(2-Naphtyl)ethenyl]-1-propylimidazole **(23)** The 0.056 g (0.0096 mol) of mixture of *cis* and *trance* isomers was obtained from 3.2 g (0.017 mol) of 3-(2-naphtyl) -2-propenpenal **29**. Yield 0.56%. mp.99°C. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, J=7 Hz), 1.61–2.04 (2H, m), 3.98 (2H, t, J=7 Hz), 6.99 (1H, d, J=9 Hz) , 7.28–7.89 (10H, m).

3-(2-Naphtyl)-2-propenpenal **29** used as starting material was prepared as follow.

3-(2-Naphtyl)-2-propenpenal **(29)** The mixture of 2-naphthaldehyde (8.25 g, 0.0528 mol), diethylphosphonoacetic acid ethyl ester (14.4 g, 0.063 mol) and potassium carbonate (14.6 g, 0.0106 mol) in dry methanol was refluxed for 5 hr. After concentrating of the mixture *in vacuo*, the residue was extracted with diethylether, following in

washing by the water and brine, and dried over sodium sulfate. After concentrating, the precipate was recrystallized from toluene. 3-(2-Naphtyl)-2-propenoic acid ethyl ester of 10.2 g (0.045 mol) was obtained (Yield 85%). 3-(2-Naphtyl)-2-propenpenal **29** was synthesized in the same way as **28** with use of ethyl 3-(2-naphtyl)-2-propenpenol instead of 4-chlorocinnam alcohol.

5-(3,4-Methylenedioxystyryl)-1-propylimidazole **(25)** Yield 15%. mp.76 °C. ^{1}H-NMR ($CDCl_3$) δ : 0.97 (3H, t, J=7 Hz), 1.61-2.04 (2H, m), 3.98 (2H, t, J=7 Hz), 6.02 (2H, s), 6.12-7.52 (7H, m). 5-(3,4-Methylenedioxystyryl)-1-propylimidazole (0.45 g, 0.0017 mol) was obtained from 1.92 g (0.0109 mol) of 3,4-methylenedioxycinnamaldehyde **30**.

3,4-Methylenedioxycinnamaldehyde **(30)** The mixture of 3,4-methylenedioxycinnam alcohol (3.81 g, 0.028 mol) and manganese dioxide (45 g, 0.52 mol) in dichloromethane was stirred for 1 day at room temperature. After filtrating, the mixture was concentrated. The residue was purified by column chromatography on silica gel. 1.92 g (0.011 mol) of pure **30** was obtained. Yield 51%. ^{1}H-NMR ($CDCl_3$) δ : 6.01 (2H, s), 6.52-5.82 (2H, m), 7.02-7.42(3H, m), 9.72 (1H, d, J=7 Hz).

2. Bioassay

Lettuce (*Lactuca sativa* L. cv. Sacramento) seeding tests were conducted by the method described previously (Kikuchi *et al.*, 1991). After 4 days cultivation, the bleating activity was estimated on a scale 0-4 according to the following ordinal categories: 0, no visual change compared with the control; 1, faint bleeching at the edges of leaves; 2, intermediate between category 1and 3; 3, small green area remaining on the leaves; 4, complete bleaching. The bleaching activity from each treatment is indicated by average result from 60 seedings.

Radish (*Raphanus sativus* L. cv. Osaka) seedlings were used to analysis of β-carotene and phytoene. Radish seeds were sterilized with 1% sodium hypochlorite for 1 hr at room temperature, washed with raining water for 2-3 hr, and soaked in deionized water for 1 days at 30 °C in dark condition. Radish seeds were germinated and grown for 4 days on two additional of filter papers, which had been treated with acetone solution (2 ml) containing various concentration of the compound and dried at room temperature, in toll Petri dishes with 20 ml distilled deionized water at 25 °C with 12 photoperiod (photosynthetically available radiation of 60 mmol·m^{-2}·s^{-1}) at relative humidity of 60%. After incubation, flesh leaves were cut off, and wiped slightly with paper. Ten grams of leaves were freeze-dried with liquid nitrogen and stored at -50 °C. The harvested leaves were taken for extraction and analysis of pigments.

3. Extraction and analysis of pigments

An extracting experiment was carried out in the dark. A chlorophyll was extracted with hot methanol at 60 °C for 15 min with shaking, and methanol solution was filtrated through a cotton batting. The chlorophyll content was determined spectctrophotometrically (663, 645 nm) using Arnon's equation (Mackinney, 1940). Carotenoids were extracted from this methanol solution as according to follows. To the methanol solution was added an equal volume of methanol containing 12% (w/w) KOH, and the solution incubated at 60 °C for additional 15 min. The filtrated extracts were partitioned into an

equal volume of diethylether/petroleum ether (1:9, v/v) and brine in a separate funnel. The water layer was extracted again with diethylether/petroleum ether (1:9, v/v). A combined diethylether/petroleum ether was washed with water 5 times and brine, dried over anhydrous sodium sulfate for 1–2 hr followed by filtering through grass filter and concentrating at room temperature *in vacuo*. To the reside was added a small volume of diethyl ether. The total carotenoids were quantitated from this extract by their absorbance at 445 nm (Schroder *et al.*, 1992).

HPLC analysis was performed as modified G. Sandmann's method (Sandmann *et al.*, 1990). The reversephase system (Shimazu ODS II, 5 mm column 25 cm × 4 cm diameter) was used with acetonitorile/methanol/2–propanole (7:1:2) at a flow rate of 1.5 ml/min. All solvents were HPLC grade (Kanto Chem. Co.). The temperature was kept at 40 °C. The eluted solution was monitored by measuring the absorbance of effluents at 445 nm and 287 nm for β–carotene and phytoene, respectably. Compounds were identified by each retention time of standard. Chlorophyll, total carotenoid, β–carotene and phytoene content in treated plants were calculated as percentage of that in the control. Each experiment was repeated four times.

4. Extraction and determination of phytoene

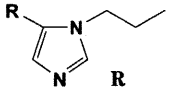
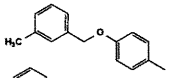
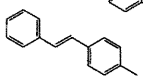
Phytoene was extracted from radish leaves (150 g) treated with fluridone by the above method. The carotenoids dissolved in a small volume of diethyl ether were fractionated into xantophylls and carotenes by passing through a small silica gel column (6 × 2 cm). The carotenes were eluted with 0.5% acetone in hexane (about 300 ml). After evaporation of the solvent at room temperature, the carotenes were dissolved in diethyl ethyl, and separated on thin layer chromatography (silica gel 60 F254; Merck c.o.). Phytoene was separated on with 0.5% ethyl ether in petroleum ether as developing solvent, the band of phytoene (Rf value; 0.45–0.55) detected UV lamp were scraped off, eluted with diethyl ether and concentrated *in vacro*. The crude phytoene was dissolved in diethyl ether and purified by the above–mentioned reverse–phase HPLC system. The structure of phytoene was identified by UV spectrum (λ_{max} at 277,286 and 298) and FD–Mass spectrum(M^+ ion at m/z 544) analysis.

RESULTS AND DISCUSSION

In our previous study, we have described that 5–phenyl–1–propylimidazole 1 and 5–(4–benzyloxyphenyl)–1–propylimidazole showed bleaching activity in lettuce seedlings. Introducing a 4–chloro and 3–methyl substituent on benzene ring (compound **2** and **3**, respectively) caused increase of the activity, while 5–alkenyl–1–propylimidazoles were inactive at even 100 ppm. The first examination was taken about series of 1–propylimidazole with an aromatic substituent on 5–position of imidazole ring (Table 1). 2–Naphthyl analog **9** showed higher bleaching activity than compound **1**, while 1–naphthyl analog **8** was inactive. 5–(3,4–Methylenedioxy)phenyl analog **10** had activity comparable to that of compound **1** at 10 ppm, but compound **10** did not cause completed bleaching at high concentration of 50 and 100 ppm. 5–Pyridyl analogs **5–7**, 5–(3,4–ethylenedioxyphenyl analog **11**, and 5–(2–indolyl) analog **12**, had no activity even at 50 ppm.

Only 1–ethyl– and 1–propyl analogs showed significant activity in both series of

Table 1. Bleaching activity of 1–propyl–5–substituted imidazoles against 4–days lettuce seedlings.

Compound		Bleaching activity* Concentration (ppm) 5	10	30	50	100
1		0	1	3	4	4
2		3	4	4	4	4
3		1	4	4	4	4
4		0	1	4	4	4
5		0	0	0	0	0
6		0	0	0	0	0
7		0	0	0	0	0
8		0	0	0	0	0
9		1	2	3	3	4
10		0	2	2	2	2
11		0	0	0	0	1
12		0	0	0	0	0

* For rating: see *Materials and Methods*

5–phenyl and 5–(4–benzyloxyphenyl)imidazoles (Yamada *et al.*, 1992). The next modification was carried out by introducing of phenylalkyl groups **13–17** at 5–position in 1–ethyl– or 1–propylimidazoles (Table 2). 5–(2–Phenethyl) and 5–phenoxymethyl analogs (**15** and **17**) had stronger activity than compound **1** at 5 ppm, however phenylalkyl series with other size of alkyl group (**13**, **14** and **16**) were less active than compound **15**. Since 5–styrlbenyl analog **4**, which we reported previously, and 5–(2–naphthyl) analog showed the bleaching activity, additional modification was made in aromatic olefinic moiety at the 5–position of the imidazole ring. 5–Cinnamyl analog **18** exhibited activity comparable to that of compound **15**, and the introduction of chlorine atom at the 3– or 4–position (**19** and **20**) extremely decreased the activity in comparison with that of compound **18**. 5–(α–Methylcinnamyl) **21** and 5–(3–furanacryl) **22** moieties were less active than compound

Table 2. Bleaching activity of 1–ethyl– and 1–propyl–5–substituted imidazoles against 4–days lettuce seedlings.

Compound	R_1	R_2	Bleaching activity* Concentration (ppm) 5	10	30	50	100
13				0	0	1	2
14				0	0	0	0
15			1	3	3	3	4
16			0	0	0	0	0
17			1	2	2	3	3
18			1	2	3	3	3
19			0	0	0	1	3
20			0	1	2	3	4
21			0	2	3	3	3
22			0	1	2	2	2
23			0	0	0	0	0
24			0	0	0	0	**
25				0	0	0	1

* For rating: see *Materials and Methods*
** not germinated

18. 5–(2–Naphthylacryl) analogs **(23)**, 5–[(3,4–methylendioxy)cinnamyl] analogs **24** and 5–biphenyl analog **25** caused no visible bleaching injury, even at 100 ppm.

The influence of three type 1,5–disubstituted imidazoles (**2, 3** and **18**), which showed bleaching activity strongly, on chlorophyll and total carotenoids was investigated by using radish seedings (Fig. 1). Compound **2** and **3** extermely reduced chlorophyll and total carotenoids contents with even a low concentration of 5 ppm, and with that more than 30 ppm respectively. The reduction rate of chlorophyll and total carotenoids by application of compound **18** was changed gently corresponding to the treated concentration, being different from those of compounds **2** and **3**. Chlorophyll contents in the seedlings

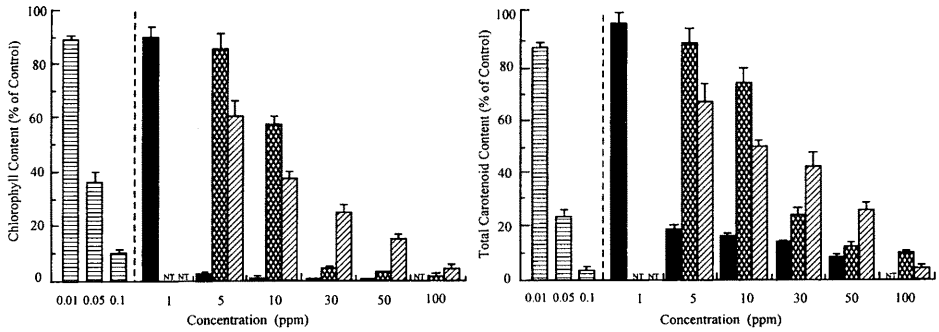


Fig. 1. Effect of 1–propyl–5–substituted imidazoles on chlorophyll and total carotenoid contents in 4–day–old Raphanus Leaves.
fluridone: ▤, compound **2**: ■, compound **3**: ▩, compound **18**: ▨.
Vertical bars represented standard division. NT showed not tested

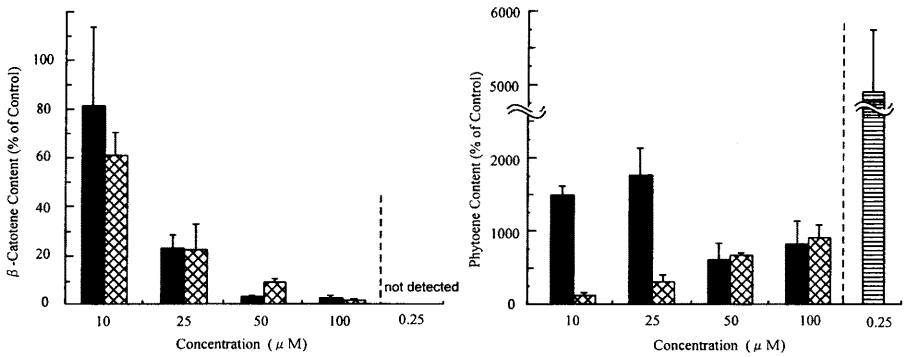


Fig. 2. Effect of 1–propyl–5–substituted imidazoles on β–carotene and phytoene contents in 4–day–old Raphanus Leaves.
fluridone: ▤, compound **2**: ■, compound **3**: ▩.
Vertical bars represented standard division.

treated with 30 ppm and 50 ppm of compound **18** were reduced to 25% and 20% respectively of that of the control, but total carotenoids contents were 45% and 30% respectively of compared with that of the control. This result suggested that bleaching symptoms caused by compound **18** was not due to inhibiting mainly carotenoid biosynthesis.

Since compound **2** and **3** reduced total carotenoids contents in radish seedlings, we examined the effect on β–carotene and phytoene contents (Fig. 2). In this determination, the concentration of treated compounds was displayed by μM, because of great difference in molecular weight of these compounds. These compounds reduced β–carotene content correlating well with the treated dosage, and at high concentration of 100μM β–carotene was hardly detected. Compound **2** and **3** caused the accumulation of phytoene in the seedlings by 700 times over as much as control at 50μM. The accumu-

lation of phytoene in the application of compound **2** at 10 and 25 μM was higher than that at 50 μM, treatment of compound **3** at 10 μM caused only slight accumulation. These results indicated that the decreasing of total carotenoids and β-carotene caused by treatment of compound **3** was not owing to inhibition of phytoene desaturation, because the degree of phytoene accumulation was small compared with that of fluridone. While compound **2** at only low concentration might inhibit phytoene desaturation moderately to decrease contents of β-carotene and total carotenoids.

It is interested that three type of 1-propyl-5-substituted imidazole cause distinct bleaching activity, and the effect of these compounds on carotenoid bioynthesis are different. More structural modification of 1,5-disubstituted imidazole and the research on target sight of these compounds might be useful for discovery of new class bleaching herbicides.

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