Original Article

Hydrogenated and Dichlorinated *N*-Phthaloyl-L-threonines and Their Dehydrated Phthalimides with Root Growth-promoting Activity for Rice Seedlings

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Hydrogenated *N*-phthaloyl-L-threonines (P-Thrs) and their dehydrated phthalimides (2-phthalimido-2-butenic acids, P-BTEAs), novel plant growth regulators, were readily synthesized. *N*-(4,5-Dichlorophthaloyl)-L-threonine and its dehydrated phthalimide also were synthesized. The synthetic P-Thrs and P-BTEAs had strong root growth-promoting activity for rice seedlings. The P-Thr activities were 1.6- to 2.2-fold the control value at 1×10^{-4} M, and the activities of the dehydrated P-BTEAs were a little higher, 2.1- to 2.6-fold the control value at the same concentration. Hydrogenation of the benzene ring of the phthaloyl group reduced root growth-promoting activity. Interestingly, none of the hydrogenated and dichlorinated P-Thrs and P-BTEAs had significant root growth-promoting activity for seedlings of lettuce, Chinese cabbage and black gram, like that of the original *N*-phthaloyl-L-threonine (P-Thr, **5a**) and its dehydrated phthalimide (P-BTEA, **6a**).

Key words: *N*-tetrahydrophthaloyl-L-threonine, 2-(tetrahydrophthalimido)-2-butenic acids, *N*-hexahydrophthaloyl-L-threonine, 2-(hexahydrophthalimido)-2-butenic acids, root growth-promoting activity, plant growth regulator.

INTRODUCTION

AC-94377 and other substituted phthalimides mimic the plant growth-regulating activity of gibberellins.^{1,2)} *N*-Phthaloyl-L-valine anilide and its related compounds are acetohydroxyacid synthetase inhibitors.³⁾ In our study of plant growth regulators, we found that non-substituted and substituted *N*-phthaloyl-L-threonines (P-Thrs, **1**) and their dehydrated phthalimides (2-phthalimido-2-butenic acids, P-BTEAs, **2**) have strong root growth-promoting activity in rice seedlings (Fig. 1).⁴⁾ The structure-activity relationships between the P-Thrs and P-BTEAs, however, have yet to be clarified. 5,6-Dichloroindole-3-acetic acid, which has two chlorine atoms in its molecule, is reported as having the strongest auxin activity.⁵⁾ Substitution of vicinal choline atoms at the 5 and 6 positions of the indole nucleus is very important for strong auxin activity.⁵⁾ El-Naggar's group has synthesized 4,5-dichlorophthaloyl-amino acids and their derivatives in their studies of the synthesis and microbial activity of halogenated

phthaloyl-aminoacids and their derivatives.^{6–8)} 4,5-Dichlorophthaloyl-L-threonine and its dehydrated phthalimide, however, have not previously been synthesized. In order to clarify the structure-activity relationships in the P-Thrs and P-BTEAs, we focused to synthesize hydrogenated P-Thrs and their dehydrated P-BTEAs in addition to dichloro-substituted P-Thr and P-BTEA. We here report the one-step synthesis and plant growth-regulating activity of hydrogenated and dichlorinated *N*-phthaloyl-L-threonines and their dehydrated phthalimides.

MATERIALS AND METHODS

1. Instrumentation

All melting points (mp) are uncorrected. IR spectra were recorded with a JASCO IR-810 spectrophotometer, and ¹H NMR spectra with a Hitachi R-22 (90 MHz) spectrometer with tetramethylsilane in acetone-*d*₆ as the internal standard. Chemical ionization (CI) mass spectra were recorded with a Hitachi M-80 spectrometer with methane as the reagent gas at 100 eV. Elemental analyses were done with a Perkin-Elmer 2400 CHN elemental analyzer.

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Fig. 1 Structure of substituted *N*-phthaloyl-L-threonine (**1**) and (*Z*)-2-phthalimido-2-butenic acid (**2**).

2. Synthetic Procedures

Hydrogenated and dichlorinated *N*-phthaloyl-L-threonines and their dehydrated phthalimido-2-butenic acids were synthesized by the following three methods.

2.1 Method A

A mixture of phthalic anhydride (**4**, 2.0 g, 9–14 mmol), L-threonine (**3**, 10–15 mmol, 1.1 mol equivalent of the anhydride), toluene (50 ml) and triethylamine (1 ml) was refluxed for about 5 hr, after which the mixture was concentrated *in vacuo* to the residual powder. Water (50 ml) and 4*N*-HCl (1 ml) were added, after which the mixture was treated three times with ethyl acetate. The combined ethyl acetate layer was washed with distilled water and saturated brine then dried over anhydrous sodium sulfate and evaporated *in vacuo* giving a pale yellow powder which was purified by silica gel column chromatography with 20% ethyl acetate in *n*-hexane. This gave polar hydrogenated or dichlorinated *N*-phthaloyl-L-threonine (**5**) and the less polar dehydrated hydrogenated or dichlorinated phthalimido-2-butenic acid (**6**). Both products were recrystallized from ethyl acetate-*n*-hexane.

2.2 Method B

A mixture of phthalic anhydride (**4**, 2.0 g, 9–14 mmol), L-threonine (**3**, 10–15 mmol, 1.1 mol equivalent of the anhydride), toluene (50 ml) and triethylamine (1 ml) was refluxed for about 3–4 hr in a flat-bottomed boiling flask fitted with a Dean-Stark moisture trap and a reflux condenser. Thereafter, the procedure was the same as in Method A.

2.3 Method C

A mixture of phthalic anhydride (**4**, 2.0 g, 9–14 mmol), L-threonine (**3**, 10–15 mmol, 1.1 mol equivalent of the anhydride) and dimethylformamide (50 ml) was refluxed for about 2 hr, then concentrated *in vacuo* yielding the residual powder. Water (150 ml) and 4*N*-HCl (30 ml) were added, after which the mixture was treated three times with diethyl ether. Thereafter, the procedure was the same as in Method A.

2.4 Spectral data of the phthaloyl-L-threonines and phthalimido-2-butenic acids

2.4.1 Spectral data of *N*-phthaloyl-L-threonine (*P*-Thr, **5a**) and (*Z*)-2-phthalimido-2-butenic acid (*P*-BTEA, **6a**)

The spectral data of these compounds are given in reference 4.

2.4.2 *N*-(3,4,5,6-Tetrahydrophthaloyl)-L-threonine (*THP*-Thr, **5b**) and (*Z*)-2-(3,4,5,6-Tetrahydrophthalimido)-2-butenic acid (*THP*-BTEA, **6b**)

5b: mp. 153–154°C; $[\alpha]_D^{20}$ –41.3° (*c*=2.0, acetone); $^1\text{H NMR}$ δ ppm: 1.25 (3H, d, *J*=6.3 Hz), 1.73–1.94 (4H, m), 2.27–2.52 (4H, m), 4.54 (1H, dq, *J*=6.0, 6.3 Hz), 4.71 (1H, d, *J*=6.0 Hz); IR ν_{KBr} cm^{-1} : 3345, 2925, 1782, 1755, 1710, 1405, 1250, 1200, 1109, 1024, 908, 725; CIMS (relative intensity, %) *m/z*: 254 (M^+ +1, 100), 236 (32), 218 (22), 192 (81), 152 (5); Found: C, 56.84; H, 6.00; N, 5.27; Calcd. for $\text{C}_{12}\text{H}_{15}\text{NO}_5$: C, 56.92; H, 5.93; N, 5.54%.

6b: mp. 146–147°C; $^1\text{H NMR}$ δ ppm: 1.75 (3H, d, *J*=6.6 Hz), 1.76–2.04 (4H, m), 2.37–2.63 (4H, m), 7.26 (1H, q, *J*=6.6 Hz); IR ν_{KBr} cm^{-1} : 3475, 2945, 1722, 1705, 1665, 1438, 1390, 1364, 1320, 1286, 1098, 1078, 885, 820, 750; CIMS (relative intensity, %) *m/z*: 236 (M^+ +1, 100), 218 (92), 137 (92), 109 (17); Found: C, 61.15; H, 5.70; N, 6.01; Calcd. for $\text{C}_{12}\text{H}_{13}\text{NO}_4$: C, 61.28; H, 5.53; N, 5.96%.

2.4.3 *cis*-1,2,3,6-tetrahydrophthaloyl-L-threonine (*cis*-THP-Thr, **5c**) and (*Z*)-2-(*cis*-1,2,3,6-tetrahydrophthalimido)-2-butenic acid (*cis*-THP-BTEA, **6c**)

5c: mp. 170–171°C; $[\alpha]_D^{20}$ –40.7° (*c*=2.0, acetone); $^1\text{H NMR}$ δ ppm: 1.15 (3H, d, *J*=6.0 Hz), 2.31–2.57 (4H, m), 3.12–3.40 (2H, m), 4.49 (1H, dq, *J*=5.7, 6.0 Hz), 4.71 (1H, d, *J*=5.7 Hz), 5.89–6.00 (2H, m); IR ν_{KBr} cm^{-1} : 3300, 2980, 1785, 1758, 1690, 1399, 1260, 1175, 1058, 910; CIMS (relative intensity, %) *m/z*: 254 (M^+ +1, 100), 236 (69), 218 (16), 192 (17), 152 (8); Found: C, 56.94; H, 6.02; N, 5.51; Calcd. for $\text{C}_{12}\text{H}_{15}\text{NO}_5$: C, 56.92; H, 5.93; N, 5.54%.

6c: colorless oil; $^1\text{H NMR}$ δ ppm: 1.61 (3H, d, *J*=7.0 Hz), 2.34–2.69 (4H, m), 3.20–3.48 (2H, m), 5.80–6.21 (2H, m), 7.20 (1H, q, *J*=7.0 Hz); IR ν_{KBr} cm^{-1} : 3460, 3040, 1780, 1711, 1660, 1417, 1391, 1370, 1315, 1269, 1200, 895, 738; CIMS (relative intensity, %) *m/z*: 236 (M^+ +1, 100), 218 (95), 188 (65), 109 (30); HRMS *m/z* (M^+): Calcd. for $\text{C}_{12}\text{H}_{13}\text{NO}_4$: 235.0844, Found: 235.0834.

2.4.4 *trans*-1,2,3,4,5,6-hexahydrophthaloyl-L-threonine (*trans*-HHP-Thr, **5d**) and (*Z*)-2-(*trans*-1,2,3,4,5,6-hexahydrophthalimido)-2-butenic acid (*trans*-HHP-BTEA, **6d**)

5d: mp. 113–114°C; $[\alpha]_D^{20}$ –39.3° (*c*=2.0, acetone); $^1\text{H NMR}$ δ ppm: 1.22 (3H, d, *J*=6.1 Hz), 1.27–1.64 (4H, m), 1.65–2.04 (4H, m), 2.43–2.69 (1H, m), 3.03–3.20 (1H, m), 4.56 (1H, dq, *J*=6.2, 6.1 Hz), 4.71 (1H, d, *J*=6.2 Hz); IR ν_{KBr} cm^{-1} : 3400, 2920, 1778, 1758, 1700, 1420,

1315, 1263, 1215, 1180, 1045, 923, 895; CIMS (relative intensity, %) m/z : 256 ($M^+ + 1$, 100), 238 (98), 220 (36), 210 (53), 154 (19); Found: C, 56.38; H, 6.77; N, 5.48, Calcd. for $C_{12}H_{17}NO_5$: C, 56.47; H, 6.67; N, 5.49%.

6d: colorless oil; 1H NMR δ ppm: 1.40–1.62 (4 H, m), 1.72 (3H, d, $J = 7.0$ Hz), 2.13–2.20 (4 H, m), 3.00–3.24 (2 H, m), 7.22 (1 H, q, $J = 7.0$ Hz); IR ν_{KBr} cm^{-1} : 3420, 2930, 1785, 1711, 1660, 1415, 1385, 1369, 1309, 1185, 895, 825, 764; CIMS (relative intensity, %) m/z : 238 ($M^+ + 1$, 100), 220 (97), 202 (33); HRMS m/z (M^+): Calcd. for $C_{12}H_{15}NO_4$: 237.1001, Found: 237.1010.

2.4.5 *cis*-1,2,3,4,5,6-hexahydrophthaloyl-L-threonine (*cis*-HHP-Thr, **5e**) and (*Z*)-2-(*cis*-1,2,3,4,5,6-hexahydrophthalimido)-2-butenic acid (*cis*-HHP-BTEA, **6e**)

5e: mp. 112–113°C; $[\alpha]_D^{20} -39.5^\circ$ ($c = 2.0$, acetone); 1H NMR δ ppm: 1.25 (3 H, d, $J = 6.4$ Hz), 1.35–1.66 (4 H, m), 1.67–2.00 (4 H, m), 2.91–3.29 (2 H, m), 4.54 (1 H, dq, $J = 6.7$, 6.4 Hz), 4.73 (1H, d, $J = 6.7$ Hz); IR ν_{KBr} cm^{-1} : 3530, 2925, 1780, 1734, 1720, 1451, 1408, 1386, 1280, 1200, 1178, 1070, 908, 819, 775; CIMS (relative intensity, %) m/z : 256 ($M^+ + 1$, 100), 238 (84), 220 (30), 154 (37); Found: C, 56.50; H, 6.77; N, 5.48, Calcd. for $C_{12}H_{17}NO_5$: C, 56.47; H, 6.67; N, 5.49%.

6e: mp. 227–229°C; 1H NMR δ ppm: 1.30–1.60 (4 H, m), 1.71 (3H, d, $J = 7.3$ Hz), 1.72–2.02 (4 H, m), 2.89–3.32 (2 H, m), 7.21 (1 H, q, $J = 7.3$ Hz); IR ν_{KBr} cm^{-1} : 3420, 2935, 2900, 2840, 1720, 1690, 1445, 1430, 1413, 1312, 1280, 1258, 1208, 922, 733; CIMS (relative intensity, %) m/z : 238 ($M^+ + 1$, 100), 220 (94), 202 (61); HRMS m/z (M^+): Calcd. for $C_{12}H_{15}NO_4$: 237.1001, Found: 237.1024.

2.4.6 *N*-(4,5-Dichlorophthaloyl)-L-threonine (4,5- Cl_2 P-Thr, **5f**) and (*Z*)-2-(4,5-dichlorophthalimido)-2-butenic acid (4,5- Cl_2 P-BTEA, **6f**).

5f: mp. 192–194°C; $[\alpha]_D^{20} -44.9^\circ$ ($c = 2.0$, acetone); 1H NMR δ ppm: 1.32 (3H, d, $J = 6.4$ Hz), 4.67 (1H, dq, $J = 7.5$, 6.4 Hz), 4.71 (1H, d, $J = 7.5$ Hz), 8.12 (2H, s); IR ν_{KBr} cm^{-1} : 3475, 2980, 1789, 1768, 1722, 1383, 1200, 1144, 745; CIMS (relative intensity, %) m/z : 322 (9), 320 (66), 318 ($M^+ + 1$, 100), 300 (22), 282 (19), 254 (85); Found: C, 45.28; H, 2.84; N, 4.40, Calcd. for $C_{12}H_9Cl_2NO_5$: C, 45.28; H, 2.83; N, 4.40%.

6f: mp. 244–245°C; 1H NMR δ ppm: 1.87 (3H, d, $J = 6.4$ Hz), 7.50 (1H, q, $J = 6.4$ Hz), 8.12 (2H, s); IR ν_{KBr} cm^{-1} : 3430, 2960, 1784, 1730, 1690, 1640, 1400, 1377, 1330, 1305, 1150, 1118, 897, 798; CIMS (relative intensity, %) m/z : 304 (10), 302 (63), 300 ($M^+ + 1$, 100), 286 (5), 284 (28), 282 (45), 258 (23), 256 (27), 254 (67), 236 (35), 201 (54); Found: C, 47.84; H, 2.47; N, 4.81, Calcd. for $C_{12}H_7Cl_2NO_4$: C, 48.00; H, 2.33; N, 4.67%.

3. Plant Materials and Bioassay

Seeds of rice (*Oryza sativa* cv. Koshihikari), lettuce (*Lactuca sativa* cv. Gokuwase-CISCO), Chinese cab-

bage (*Brassica pekinensis* cv. Kinshu) and black gram (*Vigna mungo*) all of which had been stored at 5°C in a refrigerator were used in the seedling tests.

3.1 The rice seedling test

The bioassay was done as reported previously.⁴⁾ Duplicate experiments were conducted four times.

3.2 The lettuce seedling test

The bioassay was done as reported previously.⁴⁾ Duplicate experiments were conducted three times.

3.3 The Chinese cabbage seedling test

Chinese cabbage seeds which had been washed thoroughly with running water were placed on cotton soaked in distilled water in a Petri dish ($\phi 15 \times 3$ cm) then kept for one day at 25°C in the dark in a growth chamber (Shimadzu BITEC-400L). Ten of the germinated seeds were placed on filter paper ($\phi 5.5$ cm) soaked in an aqueous solution of the test compound in a Petri dish ($\phi 6 \times 1.8$ cm) and cultured in a growth chamber maintained at 25°C under a cycle of 16-hr light and 8-hr dark. After 3 days, root and hypocotyl lengths were measured. Duplicate experiments were conducted three times.

3.4 The black gram seedling test

Black gram seeds which had been washed thoroughly with running water were placed on cotton soaked in distilled water in a Petri dish ($\phi 15 \times 3$ cm) then kept for one day at 25°C in the dark in the growth chamber. Seven of the germinated seeds were placed on filter paper ($\phi 5.5$ cm) soaked in an aqueous solution (7 ml) of the test compound in a deep Petri dish ($\phi 6 \times 6$ cm) and cultured at 25°C under a cycle of 16-hr light and 8-hr dark in the growth chamber. After 3 days, root and hypocotyl lengths were measured. Duplicate experiments were conducted three times.

RESULTS AND DISCUSSION

Hydrogenated *N*-phthaloyl-L-threonines (HP-Thrs, **5b–e**) and their dehydrated phthalimides (HP-BTEAs, **6b–e**) were readily synthesized from L-threonine (**3**) and hydrogenated phthalic anhydrides (**4b–e**) by three methods (Fig. 2). Dichlorinated *N*-phthaloyl-L-threonine (4,5- Cl_2 P-Thr, **5f**) and its dehydrated phthalimide (4,5- Cl_2 P-BTEA, **6f**) also were prepared from 4,5-dichlorophthalic anhydride (**4f**). Of the synthetic methods used, method C (coupling in DMF) gave the highest yield of phthaloyl-L-threonine (Table 1). The combined P-Thrs and P-BTEAs yield was moderate (52–63%). Under the continuous removal of water in method B, the dehydrated phthalimide yield increased. Dehydrated phthalimides (**6**) were produced due to the loss of water from the mother compounds, the *N*-phthaloyl-L-threonines. The (*Z*)-stereochemistry of the dehydrated phthalimides was determined by comparing the chemical shifts of their olefinic protons with those of 2-phthalimido-2-butenic acid (P-BTEA, **6a**), the stereochemistry of which has been determined by X-ray analy-

Fig. 2 Synthesis of hydrogenated and 4,5-dichlorinated *N*-phthaloyl-L-threonines and (*Z*)-2-phthalimido-2-butenic acids.

Table 1 Phthaloyl-L-threonines (**5**) and phthalimido-2-butenic acids (**6**) ratios obtained by three methods.^{a)}

Anhydride	Method ^{a)}	Product (% yield) ^{b,c)}	
		5	6
Phthalic anhydride (4a)	A	24	9
	B	21	30
	C	38	14
3,4,5,6-Tetrahydrophthalic anhydride (4b)	A	24	10
	B	15	28
	C	45	10
<i>cis</i> -1,2,3,6-Tetrahydrophthalic anhydride (4c)	A	35	9
	B	19	38
	C	48	14
<i>trans</i> -1,2,3,4,5,6-Hexahydrophthalic anhydride (4d)	A	* ^{d)}	* ^{d)}
	B	9	18
	C	45	15
<i>cis</i> -1,2,3,4,5,6-Hexahydrophthalic anhydride (4e)	A	15	5
	B	10	23
	C	46	16
4,5-Dichlorophthalic anhydride (4f)	A	18	14
	B	5	30
	C	49	14

^{a)} See MATERIALS AND METHODS. ^{b)} Isolated yield. ^{c)} Phthalic acids also were produced in these methods. Phthalic acid yield, 10–47%. ^{d)} Not done.

sis of its methyl ester.⁹⁾

The biological activities of the synthetic hydrogenated and dichlorinated *N*-phthaloyl-L-threonines and their dehydrated phthalimides were measured in rice, lettuce, Chinese cabbage and black gram seedlings. The findings for rice seedlings are shown in Fig. 3. All the hydrogenated and dichlorinated *N*-phthaloyl-L-threonines had strong root growth-promoting activity for rice seedlings, 1.6- to 2.2-fold the control value at 1×10^{-4} M. Interestingly, all the dehydrated phthalimides (P-BTEAs, **6**) produced by the loss of water from the *N*-phthaloyl-L-threonines (**5**) had stronger activities than

the threonines, 2.1- to 2.6-fold the control value at 1×10^{-4} M. Hydrogenation of the benzene ring of *N*-phthaloyl-L-threonine (**5a**) and of 2-phthalimido-2-butenic acid (**6a**) somewhat reduced root growth-promoting activity, except for THP-Thr (**5b**). In contrast, the introduction of two vicinal chlorine atoms to the aromatic ring of *N*-phthaloyl-L-threonine (**5a**) somewhat increased root growth-promoting activity in rice seedlings. The two chlorine atoms in 2-dichlorophthalimido-2-butenic acid (**6f**), however, decreased this activity. Hydrogenated *N*-phthaloyl-L-threonines (HP-Thrs, **5b–e**) and their dehydrated

Fig. 3 Effects of hydrogenated and 4,5-dichlorinated *N*-phthaloyl-L-threonines and (*Z*)-2-phthalimido-2-butenic acids on rice seedlings.

■ : 1×10^{-3} M, ■ : 1×10^{-4} M, ■ : 1×10^{-5} M, ■ : 1×10^{-6} M, * : necrosis.

Fig. 4 Effects of hydrogenated and 4,5-dichlorinated *N*-phthaloyl-L-threonines and (*Z*)-2-phthalimido-2-butenic acids on lettuce seedlings.

■ : 1×10^{-3} M, ■ : 1×10^{-4} M, ■ : 1×10^{-5} M, ■ : 1×10^{-6} M, * : necrosis.

phthalimides (HP-BTEAs, **6b–e**) had very strong root growth-promoting activity at 1×10^{-4} M, but this activity decreased abruptly at 1×10^{-5} M, unlike that of P-Thr, 4, 5-Cl₂P-Thr and their dehydrated phthalimides (P-BTEA and 4,5-Cl₂P-BTEA). This indicates that hydrogenated P-Thrs and P-BTEAs have a very narrow concentration range for root growth-promoting activity.

Of the tetrahydro-phthaloyl-L-threonines and their dehydrated phthalimides, THP-Thr (**5b**, 3,4,5,6-tetrahydro-P-Thr) and its dehydrated THP-BTEA (**6b**) had slightly stronger activity than *cis*-THP-Thr (**5c**, *cis*-1,2,3,6-tetrahydro-P-Thr) and its dehydrated *cis*-

THP-BTEA (**6c**). Of the hexahydro-phthaloyl-L-threonines, *trans*-HHP-Thr (**5d**) had the same root growth-promoting activity as *cis*-HHP-Thr (**5e**). Of the hexahydro-phthalimides, the growth-promoting activity of *trans*-HHP-BTEA (**6d**) was the same as that of the *cis*-isomer (**6e**).

At high concentration of 1×10^{-3} M, the hydrogenated P-Thrs and P-BTEAs, THP-Thr (**5b**) and THP-BTEA (**6b**) caused severe root necrosis. *cis*-THP-Thr (**5c**) and its dehydrated *cis*-P-BTEA (**6c**) and the *trans*- and *cis*-hexahydro compounds (**5d**, **5e**, **6d**, **6e**), however, did not cause root necrosis, indicating that hydrogenation at

Fig. 5 Effects of hydrogenated and 4,5-dichlorinated *N*-phthaloyl-L-threonines and (Z)-2-phthalimido-2-butenic acids on Chinese cabbage seedlings.

■ : 1×10^{-3} M, ■ : 1×10^{-4} M, ■ : 1×10^{-5} M, ■ : 1×10^{-6} M.

Fig. 6 Effects of hydrogenated and 4,5-dichlorinated *N*-phthaloyl-L-threonines and (Z)-2-phthalimido-2-butenic acids on black gram seedlings.

■ : 1×10^{-3} M, ■ : 1×10^{-4} M, ■ : 1×10^{-5} M, ■ : 1×10^{-6} M.

the 1,2- positions of *N*-phthaloyl-L-threonine (P-Thr, **5a**) and its phthalimide (P-BTEA, **6a**) reduces root growth inhibition in rice seedlings. Dichlorinated P-Thr (**5f**) and P-BTEA (**6f**), however, also caused severe root necrosis at 1×10^{-3} M. As to the shoot growth of rice seedlings, THP-Thr (**5b**), THP-BTEA (**6b**), *cis*-HHP-Thr (**5e**) and *cis*-HHP-BTEA (**6e**) somewhat promoted it. Interestingly, *trans*- and *cis*-hexahydro-P-Thrs (**5d**, **5e**) and their P-BTEAs (**6d**, **6e**) did not inhibit shoot growth. This indicates that complete hydrogenation of the aromatic ring in *N*-phthaloyl-L-threonine completely

removes inhibition of shoot growth in rice seedlings.

Results for lettuce seedlings are shown in Fig. 4. None of the hydrogenated P-Thrs and P-BTEAs or the dichlorinated P-Thr and P-BTEA had significant root and hypocotyl growth-promoting activity. At high concentration (1×10^{-3} M), all these compounds caused severe root necrosis as did the original P-Thr (**5a**) and P-BTEA (**6a**). *cis*-THP-Thr (**5c**), *cis*-HHP-Thr (**5e**), *cis*-HHP-BTEA (**6e**) and the dichlorinated compounds (**5f**, **6f**) also caused severe hypocotyl necrosis. The other Thrs (**5b**, **5d**) and P-BTEAs (**6b**, **6c**, **6d**) moderately

inhibited hypocotyl growth as did P-Thr (**5a**) and P-BTEA (**6a**). The introduction of two chlorine atoms to the P-Thr (**5a**) molecule produced severe root and hypocotyl necrosis at 1×10^{-3} M.

Results for Chinese cabbage seedlings are shown in Fig. 5. Only two THP-BTEAs (**6b**, **6c**) had strong root growth-inhibitory activity at 1×10^{-3} M like that of the original dehydrated P-BTEA (**6a**). Unlike in rice seedlings, THP-BTEA (**6b**) had moderate root growth-inhibitory activity at 1×10^{-4} M. The other compounds did not have root and hypocotyl growth-promoting (or -inhibiting) activity.

None of the P-Thrs and P-BTEAs had significant root and hypocotyl growth-promoting (or -inhibiting) activity for the black gram seedlings (Fig. 6).

Hydrogenated *N*-phthaloyl-L-threonines and their dehydrated phthalimides had strong root growth-promoting activity only for rice seedlings but had no significant root growth-promoting activity for lettuce, Chinese cabbage and black gram seedlings, indicating that the strong root growth-promoting activity of *N*-phthaloyl-L-threonines and their dehydrated phthalimides is specific only to rice seedlings. The characters of these P-Thrs and P-BTEAs differ from the character of 4,4,4-trifluoro-3-(3-indolyl)butyric acid (TFIBA) which had root growth-promoting activity for all four seedling types: rice, lettuce, Chinese cabbage and black gram.¹⁰⁻¹⁴⁾

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要 約

イネ芽生えに対して根の成長促進活性を有する水素化またはジクロロ化された *N*-フタロイル-L-スレオニン類およびそれらの脱水フタルイミド類

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新規植物成長調節物質, 水素化とジクロロ化 *N*-フタロイル-L-スレオニン類 (P-Thrs) およびそれらの脱水フタルイミド類 (2-フタルイミド-2-ブテン酸, P-BTEAs) は容易に合成され, イネ芽生えに対して強い根の成長促進活性を示した。P-Thr 類の成長促進活性は 1×10^{-4} M で対照区の 1.6~2.2 倍であり, 一方脱水 P-BTEA 類の成長促進活性は P-Thr より少し強く対照区の 2.1~2.6 倍であった。また, フタロイル基のベンゼン環の水素化は根の成長促進活性を減少させた。さらに興味あることに水素化或いはジクロロ化 *N*-フタロイル-L-スレオニン類 (P-Thrs) 及びそれらの脱水フタルイミド類は, レタス, 白菜やブラック・マッペに対しては有意な根の成長促進活性を示さなかった。これらの結果は, P-Thr や P-BTEA 類はイネ芽生えに対して特異的に根の成長促進活性を有することを示した。