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Note

Effect of Optically Active Ethyl 2-Phthalimidooxypropionate on the Growth of Cress, *Lepidium sativum*

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The effects of 2-phthalimidooxyalkanoic acid derivatives on the germination and root-growth of cress were examined. Since 2-phthalimidooxypropionates were most effective, the optically active ethyl esters were prepared. As the result of biological testing, the (*S*)-(−)-isomer exhibited stronger activity than the (*R*)-(+)-isomer. This result is contrary to those from commercial herbicides with similar structures, phenoxy- and oxyphenoxy-propionate-type compounds, where the (*R*)-isomers are generally known to be the active principles.

Key words: phthalimidooxy derivatives; 2-phthalimidooxypropionate; optical activity; cress test; plant growth inhibition

2-Aminooxyacetic acid has been found to have interesting biological activities toward higher plants, such as the inhibition of glyoxylate reductase in photorespiration[1] and the inhibition of aminocyclopropanecarboxylic acid synthetase in ethylene production.[2] Furthermore, its derivative, L-α-aminooxy-β-phenylpropanoic acid, is an extremely potent competitive inhibitor of phenylalanine ammonia-lyase both *in vitro* and *in vivo*.[3] Monoacyl-derivatives of aminooxyacetic acid with broad structural variation have been widely investigated for their antibacterial and herbicidal activities.[4] As to its diacyl derivatives, the inhibitory effects of compounds with maleimide and phthalimide groups on ethylene production have been described,[5] but not any other activities. Since we are interested in other biological activities of such phthalimidooxy-type derivatives, we prepared some new derivatives and examined their effects on the germination and root-growth of cress, *Lepidium sativum*. Furthermore, we investigated the effects of optical isomers of the most active derivative.

Esters of 2-phthalimidooxyalkanoic acids were prepared by the reaction of *N*-hydroxyphthalimide (Tokyo Kasei Co.) with various bromoalkanoates or 2-tosylalkanoates similar to the method reported in the literature.[5] As a general procedure, to a DMF solution of *N*-hydroxyphthalimide and 2-bromoalkanoate, triethylamine in DMF was added dropwise while stirring at 0°C. After further stirring at room temperature for 1 day, the reaction mixture was diluted with water and extracted with Et_2O. The organic layer was successively washed with 2 N HCl, aq. sat. $NaHCO_3$, and brine. After drying over anhydrous Na_2SO_4, the solvent was removed under reduced pressure. The residue was purified either by silica gel chromatography or by recrystallization, using appropriate solvents. The structures of the obtained compounds were confirmed by NMR spectrometry (JEOL JMX-270) and elemental analyses. Specific rotation was measured by a Horiba SEPA-200 polarimeter.

The effects of synthetic compounds on the germination and root-growth of cress, *Lepidium sativum*, were evaluated according to the general procedures described previously.[6,7] As a result of the test (Table 1), compounds **1** and **2** inhibited germination completely at a concentration of 100 ppm, but this activity was not observed at a lower concentration. The other compounds had weak or no inhibitory effect. In the growth test, compound **1** also exhibited complete inhibition at 100 ppm. Furthermore, the introduction of a methyl group at the 2-position of **1**, compound **2**, increased the activity up to 68% inhibition, even at 1 ppm. The introduction of one more methyl group at the 2-position of **2**, compound **3**, and elongation of the alkyl group, compounds **4** and **5**, decreased the activity. Compounds with an aromatic group as the substituent, **6** and **7**, exhibited weak activity. As to the substituent at the 2-position, one methyl group was found to be the most effective. To compare the effects, compounds **8** and **9** were tested at the same time. Since they were less effective, both the N-O bond and the ethoxycarbonyl groups were important for the activity.

In order to examine the effect of the ester moiety of **2** on the activity, several esters with the alcoholic moiety of $C_{1\sim5}$, both normal and branched, were prepared and tested. As a result (Table 2), the potency of esters with normal alcohols was almost the same as that of **2**. Those with *sec*-alcohols, **12** and **14**, seemed to be less effective, and *tert*-butyl ester **15** had no effect at the same concentration. Considering the difficulty in the hydrolysis of such esters, especially in **15**, the process might be necessary to express activity after being moved into a plant, as has been reported for the commercial herbicide, fluazifop-butyl.[8]

2-Phthalimidooxypropionates were found to be the most potent compounds in their inhibitory effect on plant growth, and the effect of the configuration at the 2-position of those compounds on the activity is very interesting. So, (*R*)-(+)-**2** and its (*R*)-(−)-isomer were prepared from (*S*)-(+)-lactic acid (Wako Chemical Co.) and from (*R*)-(+)-2-bromopropanoic acid (Tokyo Kasei Co.), respectively, by the procedure already de-

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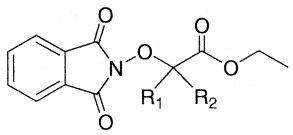
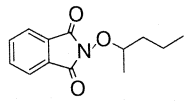
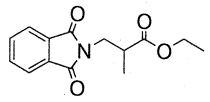
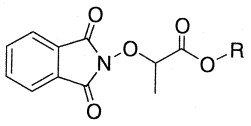
Table 1. Effects of 2-Phthalimidooxyalkanoates on the Germination and Root-Growth of Cress, *Lepidium sativum*[a]



No.	R_1	R_2	Inhibition (%) Germination Conc. (ppm) 100	10	Root-growth Conc. (ppm) 100	1
1	H	H	100	0	100	0
2	CH_3	H	100	0	100	68
3	CH_3	CH_3	60	0	95	0
4	CH_3CH_2	H	80	0	94	0
5	*n*-C_6H_{13}	H	0	—[b]	72	0
6	Ph	H	0	—	60	0
7	$PhCH_2$	H	0	—	70	0
8			0	—	56	0
9			0	—	41	0

[a] Twenty cress seeds were used for each test, and all tests were duplicated.
[b] Not tested.

Table 2. Effect of the Ester Moiety of **2** on the Root-Growth of Cress



No.	R	Inhibition (%) Conc. (1 ppm)
10	CH_3	64
11	*n*-C_3H_7	64
12	*i*-C_3H_7	51
13	*n*-C_4H_9	59
14	*sec*-C_4H_9	42
15	*tert*-C_4H_9	1
16	*n*-C_5H_{11}	64

scribed. The analytical data for each isomer are as follows: (*R*)-(+)-**2**, white crystals recrystallized from 2-propanol; mp 72°C; $[\alpha]_D^{25}$ +90.09° (c 2.22, $CHCl_3$); ^{1}H-NMR δ ppm: 1.29 (3H, t, J=7.17 Hz, -CH_2CH_3), 1.65 (3H, d, J=6.71 Hz, -$CHCH_3$), 4.18 (2H, m, -OCH_2CH_3), 4.88 (1H, q, J=6.81 Hz, -$CHCH_3$), 7.74–7.93 (4H, m, aromatic *H*); ^{13}C-NMR δ ppm: 169.7, 163.3, 134.6, 128.9, 123.7, 81.3, 61.7, 16.3, 14.0. *Anal.* Found: C, 59.54; H, 5.02; N, 5.35%. Calcd. for $C_{13}H_{13}O_5N$: C, 59.31; H, 4.98; N, 5.32%. (*S*)-(−)-**2**, white crystals; $[\alpha]_D^{25}$ −75.15° (c 2.00, $CHCl_3$). *Anal.* Found: C, 59.37; H, 4.98; N, 5.31%. Calcd. for $C_{13}H_{13}O_5N$: C, 59.31; H, 4.98; N, 5.32%. The ^{1}H- and ^{13}C-NMR data were the same as those of the (*R*)-(+)-isomer. The optical purity of each isomer was determined on the basis of each ^{1}H-NMR spectrum measured with the shift reagent, Eu(TFC)$_3$, in $CDCl_3$ by a JEOL JMX-270 instrument. When the shift reagent was added, the peaks due to the alcoholic methyl and carboxylic methyl of (*R*)-**2** were shifted more downfield than those of (*S*)-**2**. The optical purities of (*R*)-(+)- and (*S*)-(−)-**2** were estimated to be 96% ee and 80% ee, respectively.

Table 3. Effect of Optically Active **2**[a] on the Root-Growth of Cress

Sample	Growth Inhibition (%) Conc. (10^{-6} M) 3.8	1.1	0.38
(*R,S*)-**2**	68	33	14
(*S*)-(−)-**2**	72	49	35
(*R*)-(+)-**2**	14	5	0

[a] Tested by using the compounds with an optical purity of 80% ee.

In the growth-inhibiting test, using racemic and each enantiomer of **2**, the activity of the (*S*)-(−)-isomer was always stronger than that of the racemate or (*R*)-(+)-isomer, and strong correlation was observed between the content of the (*S*)-isomer and the growth-inhibiting activity (Table 3). The values of IC_{50} for (*S*)-**2** with 80% ee and the racemate were estimated to be 1.4×10^{-6} M and 2.7×10^{-6} M, respectively. This result suggests that optically pure (*S*)-**2** was approximately two-fold more active than the racemate, whereas its (*R*)-isomer had low activity or was inactive.

It is clarified that the active principles of phenoxy- and oxyphenoxypropionic acid derivatives, well-known herbicides used in practice, were their (*R*)-isomers, although their modes of action were different;[9-12] the former is known as an auxin-type herbicide, while the latter inhibits acetyl-CoA carboxylase with the resulting inhibition of fatty acid biosynthesis. Considering the structural similarity of **2** to those herbicides, the contrary result obtained in this experiment is very interesting. It is also interesting that the most active compound in this experiment was different from that in the inhibition test of ethylene production, where **1** was more effective than **2**.[5] Although the mode of action of the compounds described in this paper is not clear in detail, they might have abscisic acid-like activity, since the test used in this experiment is one of assay methods for abscisic acid analogs.[6]

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